ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS OF STRESSFUL CONDITIONS IN THE COMMON FROG *RANA TEMPORARIA*

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde

(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

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Zürich, 2012

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS OF STRESSFUL CONDITIONS IN THE COMMON FROG *RANA TEMPORARIA*

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CONTENTS

GENERAL INTRODUCTION

General Introduction

The interaction between an organism and its environment lies at the core of ecology and evolution. In fact, every organism during its entire lifetime is exposed to its surrounding environment. Environment, in this sense, represents the combination of every influence experienced by the organism, be it biotic or abiotic in nature. Stressful environmental conditions, understood as external factors that impair Darwinian fitness of an organism (Sibly and Calow 1989), are omnipresent in nature and affect biological systems at various levels, from individuals through populations and species to ecosystems (Hoffmann and Parsons 1991).

Direct effects of stressful conditions include all effects at an individual level, e.g. on behavior, growth rates, development, and other traits. Direct effects on individual fitness can have important implications for species conservation and management. Moreover, stressors can also act indirectly by affecting the genetic composition of populations, which may have implications for the likelihood of microevolutionary responses to stress. Evolutionary adaptation to stress is possible if genotypes in the population differ in fitness under stressful conditions. Specific traits can evolve to improve performance under stress if there is genetic variation in traits that affect fitness (Freeman and Herron 2007). Stress could indirectly alter the process of adaptation in multiple ways. First, stress can either increase or decrease the quantity of genetic variation in a population by several mechanisms (Hoffmann and Merilä 1999). Moreover, the decline in individual fitness may cause a decline in population size, enhancing the strength of genetic drift and thereby decreasing overall variation in the genome (Hedrick and Kalinowski 2000). If a stressor causes populations to decline below a certain limit, various processes can act to prevent adaptation and the likelihood of extinction can increase (Burger and Lynch 1995; Lynch et al 1995; Willi et al 2006). The nature and strength of a stressor can play a central role in determining the fate of a population (Hoffmann and Parsons 1991).

Stressors can broadly be separated into natural and novel stressors for a given organism. Examples of natural stressors include those that are associated with competition for resources, predation, diseases, and parasites, and that have been encountered during an organism's evolutionary history. Populations, species and ecosystems were able to evolve in their presence. Novel stressors, however, are factors that have not been experienced by an organism in its evolutionary history, and have recently been introduced into the natural environment, or are

being encountered through range expansion. Environmental contamination, habitat destruction, or alien species invasions are examples of novel stressors. If a population experiences a novel stressor, stress responses – on the phenotypic as well as genetic level – can differ dramatically from responses to natural stressors (Holloway et al 1990, Hoffmann and Parsons 1991). Hence, the evolutionary history of exposure to stress should be considered when studying the interaction between organism and environment.

In recent times, both novel and natural stressors have been strongly affected by increasing rates of environmental change due to anthropogenic impact (Jones et al 2001). Confronted with largely changed selection regimes, populations and species have failed to adapt to those new environmental conditions, and have gone extinct over the last decades at unprecedented rates (May 2010). The recent loss of populations and extinctions of species have led to the perception that we are witnessing an extinction period that matches the five mass extinction events during the Earth's history (Wake and Vredenburg 2008). Research effort should hence be directed at understanding the nature of stressors, and their direct and indirect effects on natural organisms, populations and species.

This thesis aims to contribute to our understanding of the effects of stressful environmental conditions, and looks at different aspects of the impact of stress on individual and population levels using an amphibian species as a study system. Amphibian populations are globally declining, and extinction rates of amphibians may be as high as 211 times the background rate of extinction during the Holocene (McCallum 2007). Potential causes for amphibian declines are manifold, including habitat destruction, pollution, introduced exotic species and diseases, and interactions between novel and naturally occurring stressors (Blaustein et al 2011). The life stage that is particularly sensitive to environmental conditions is the amphibian larval stage, the early aquatic developmental stage that takes place in freshwater puddles, ponds and lakes in most species. Freshwater ecosystems are currently influenced by all classes of stressors, including changes in water chemistry, and the physical environment (Altshuler et al 2011), ultimately affecting larval stages of most amphibian species.

This critical developmental stage of amphibians is particularly well suited to study the effects of stressful environments, as amphibian larvae allow for large-scale experiments suitable for ecological as well as evolutionary studies. Their experimental environment can easily be

controlled and manipulated, allowing the study of the effects of different kinds of stressors. For all studies presented here, I used the common frog *Rana temporaria* as a study species. This is the most common amphibian species in Switzerland (Meyer et al 2009), and is currently not considered to be endangered.

Thesis outline

I. Experiments with stress - can I bring nature into the lab?

Experiments can teach us about the world as long as the experimental conditions realistically reflect what happens in nature. When assessing the effects of stressful conditions on natural organisms, it is of particular importance that the insights gained from the experiment can be translated to natural conditions. Discrepancies in the outcome of laboratory and field studies are very common, including the field of stress research (see for example chapter 3), emphasizing the importance of evaluating the realism of the venues commonly used in experimental ecology and evolution.

In my first chapter I set out to compare the outcome of the same ecological experiment performed in the three most commonly used venues in experimental ecology and evolutionary biology: the laboratory, mesocosms, and the field. Mesocosms combine characteristics of the lab with those in nature, and are commonly used to create a semi-natural environment for controlled experimentation. I also aimed to weigh realism against precision and replication across all venues in order to evaluate which specific needs of the experimenter are met by which types of experimental setting. The comparison of the outcomes between the three experimental venues enhances our understanding of whether and how much experimental outcomes are influenced by the venues we use, and which of the venues best reflects processes in nature. Also, this experiment set the stage for my later research, as I was able to determine which experimental venue was best suited to study the direct and indirect effects of stress.

II. Environmental stress and ecology: implications for conservation

In the age of global ecosystem changes, studies of direct effects of stressors on individual fitness become essential for our endeavour to shed light upon the causes of population declines and species extinctions. Findings in this field of research can have immediate implications for nature conservation and the protection of species and ecosystems (e.g. Geeraerts and Belpaire 2010, Blaustein et al 2011, Moron et al 2012). A prominent trend is the widespread contamination of the environment with chemical compounds targeted for agricultural or other human use (e.g. pesticides, nitrogen, and salts). Ecotoxicological research regularly demonstrates detrimental effects of commonly used chemical compounds on fitness-related traits and life history of non-target organisms (e.g. Relyea 2005, Cresswell 2011) – effects that are not assessed through routine toxicity testing before the marketing of chemical compounds targeted for agricultural usage (for a discussion see Stark and Banks 2003).

One chemical compound that has received relatively little attention for its detrimental effects on the environment is road salt, used as the main road deicer during winter in North America and Europe. In Switzerland, thousands of tons of road salt are dispersed onto the road system every year (ASTRA, Bundesamt für Strassen, www.astra.ch), eventually running off as solutes that may contaminate wetlands, streams and drinking water.

Changes in environmental salinity can be harmful to wildlife and ecosystems. When facing unfavourable environmental conditions, an organism engages in an integrated response to counter the harmful effects of stress. This response involves all levels of functional complexity within the organism, i.e. molecular, cellular and physiological (Hoffmann and Parsons 1991, Barnosky 2009). The stress response can be metabolically costly, as it may deflect energy from normal metabolic processes. Ultimately, organismal fitness and fitness-related traits such as growth rate, development time and behavior might be affected when stressful conditions are encountered. In the second chapter, I investigated the direct effects of salt contamination of different concentrations on tadpole life history traits and behaviour. The results of this research not only have the potential to stir up discussions around the usage and application rates of road salt, but can also lead to regulatory consequences for environmental management.

III. Environmental stress and evolution: how does adaptive potential change under stress?

Environmental stressors not only have direct effects on individuals and populations, but can also be seen as an evolutionary force. To date, the evolutionary impacts of environmental stress are not entirely clear, and it is a major challenge to predict the implications of stressful conditions for the evolutionary dynamics and future performance of populations (Hoffmann and Merilä 1999, Millien et al 2006).

Two kinds of influences of stressful conditions have been extensively studied, and both ascribe a potentially important role to stress. First, a decline in individual fitness due to the experience of stressful environmental conditions might lead to a reduced potential rate of population growth and may cause a decline in population size. If this proceeds very far, selection in favor of beneficial or against detrimental alleles is overruled by genetic drift. Hence, adaptation to new environmental conditions can be prevented, and especially small and isolated populations can be driven towards extinction (Willi et al 2006). This mechanism links the direct effects of environmental stress outlined in my second chapter to its evolutionary consequences. Second, environmental stress may impose selection by moving populations away from their adaptive optima. This process presumably accelerates the rate of evolution, favoring shifts in morphology, physiology and behavior, potentially leading to new adaptations (Hoffmann and Hercus 2000).

A third influence of stressful conditions, about which relatively little is known, deals with the question of how stressful conditions affect the amount of genetic variation present in a population and how this relates to the evolutionary potential of that population.

The heritability of a trait (h^2), estimating the fraction of additive genetic variance (V_A) relative to the total phenotypic variation (V_P), provides a measure of the evolutionary potential of a phenotypic trait ($h^2 = V_A / V_P$). Only if some of the variation in a trait is heritable, and natural selection is at work, an evolutionary response can occur. The amount of variation attributable to genotypic differences, however, can differ among environments, creating condition dependency of the evolutionary potential of a trait (Hoffmann and Merilä 1999, Badyaev 2005). As yet there is no consensus on whether a stressful environment leads to increased or decreased trait heritabilities, resulting from discrepancies in the outcome of laboratory and field studies. Some characteristics of both laboratory and field studies have been suggested to be important when

explaining the differences in outcome among the two venues, including differences in novelty of the applied stressors.

In the third chapter, I assessed how genetic variation and evolutionary potential of fitness-related phenotypic traits are affected by stressful environmental conditions under the aspect of the novelty of the applied stressors. I exposed tadpoles of a large number of different families to environmental conditions representing novel and familiar stressors, and quantified how families differed in behavior, life-history and fitness-related traits under each of the stressors. The results contribute to our understanding of how populations are influenced by stressful conditions, and whether and how stressors of different qualities shape the adaptive potential of populations in a changing environment.

IV. Multiple stressors along a habitat gradient – searching for an intraspecific fitness trade-off

In principal, no population of an organism is able to evolve an optimal phenotype to all selective challenges at once. In heterogeneous environments, a trade-off can arise involving traits that confer high fitness under one environment, but are costly under another environment. The idea of fitness trade-offs is central to most theories for the evolution of ecological specialization, and is believed to explain processes determining community structure, species distributions and specialization to certain types of habitat (Fry 1996).

One of the trade-offs believed to limit species distribution and to structure communities of various classes of organisms is shaped by the interaction between predation and competition along freshwater habitat gradients (Wellborn et al 1996). This predation-competition trade-off occurs when traits that enhance competitive abilities of a species restrict its ability to avoid being killed by predators, and can be mediated for example through levels of behavioral activity: tadpoles that show relatively high levels of behavioural activity are known to be good competitors, when compared to tadpoles that are relatively inactive; however, the likelihood being encountered by a predator increases with increasing activity, and relatively active tadpoles are more likely to be predated upon. Anuran species are known to replace each other along a habitat gradient ranging from permanent ponds, where predator densities are high and competition for resources is low, to temporary puddles that contain fewer or no predators but

higher densities of competitors (Wellborn et al 1996, Van Buskirk 2003). Amphibian species occupying temporary waters are known to be more vulnerable to predators, but in general superior competitors compared to permanent pond species (Woodward 1982, Lawler 1989).

In my fourth chapter I set out to assess whether the predation-competition trade-off is present among genotypes of a population that inhabits a region where individuals may encounter both high densities of predators as well as competitors. In theory, a population could yield genotypes that are specialized in avoiding predators, as well as genotypes that are particularly well suited to compete for resources. Just as it is being found among closely related species, genotypes that are good competitors are expected to be bad at avoiding predation, and a trade-off will appear as a negative genetic correlation of the traits that mediate the predation-competition trade-off.

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INFLUENCE OF EXPERIMENTAL VENUE ON PHENOTYPE: MULTIPLE TRAITS REVEAL MULTIPLE ANSWERS

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Functional Ecology (2012) 26 (2): 513-521

Summary

1. Experiments in ecology occur in the laboratory, mesocosm, or field. The choice of venue can influence the outcome and may be associated with trade-offs involving realism and precision.

2. We evaluated these trade-offs in an experiment measuring effects of venue on larval traits of *Rana temporaria* tadpoles. The design included lab, mesocosm, and field venues, crossed with two treatments (presence and absence of caged *Anax imperator* dragonfly larvae). Realism of venues was evaluated by comparing experimental with wild tadpoles.

3. Venue influenced nearly every trait we measured, but some were more sensitive to venue than others. Larval and metamorphic performance, external morphology, and predator-induced plasticity in many traits varied among venues, while behavior was less dependent on venue. Tadpoles in mesocosms were most similar to those in field enclosures and the wild, although the phenotypic response to predation risk was greatest in the mesocosm venue. The laboratory environment triggered highly distinctive morphology. Precision was not higher in the laboratory than in other venues.

4. This study suggests that both constraints and research questions must be considered when choosing an appropriate experimental venue.

Introduction

Discussions about experimental venue have been a part of ecology for decades (Diamond 1986; Hairston 1989). The main focus has been a series of well-accepted trade-offs associated with field and laboratory venues, involving realism, control, precision, and replication. However, as the topic of venue itself has become an object of experimental study, some of the old paradigms have been overturned and new insights into the relevant trade-offs have emerged. For example, controlled experiments in laboratory containers or mesocosms do not in general yield larger effect sizes than field studies, and lab experiments do not necessarily have greater precision (Weigensberg & Roff 1996; Skelly & Kiesecker 2001; Bancroft, Baker & Blaustein 2007). On the other hand, it does appear that experimental designs implemented under more controlled mesocosm or lab conditions are more complex and better replicated, and effect sizes can be larger in the laboratory for certain types of manipulations (Skelly & Kiesecker 2001; Bell, Neill & Schluter 2003).

Some of these insights emerge from reviews and meta-analyses of experiments employing different kinds of venues (Petersen, Cornwell & Kemp 1999; Skelly & Kiesecker 2001; Bell *et al.* 2003; Bancroft *et al.* 2007). One problem with this approach is that differences among venues in the original experiments are confounded with other features that vary among studies, including organisms, geographical localities, investigators, and numerous methodological details (Chalcraft, Binckley & Resetarits 2005). What is needed are explicit manipulations of venue that hold all else constant as far as possible (Skelly 2002). Our goal here was to gauge the effects of three types of venue on larval phenotypes of *Rana temporaria*, a European amphibian that is a frequent research subject in experimental ecology. We observed behavior, external morphology, larval and metamorphic performance, and plasticity in these traits between two experimental treatments (presence and absence of predation risk), carried out in three different experimental venues. Our results are important for interpreting the ecological literature, for choosing appropriate venues in future studies, and for weighing trade-offs among realism, control, precision, and replication.

Materials and methods

The experiment had a complete factorial design consisting of three venues crossed with two treatments. The treatments were presence and absence of non-lethal dragonfly larvae, and the three venues were laboratory, outdoor mesocosm, and field enclosure. Each of the three venue types was represented by two independent realizations of the venue, here called "settings" and treated as an additional factor nested within venue (Table 1). The two laboratory settings were small plastic bins (volume 1.1 L, containing 1 tadpole) and large plastic bins (5.2 L, 5 tadpoles). In the mesocosm venue, the two settings were small plastic tubs (80 L, 12 tadpoles) and large fiberglass stock tanks (675 L, 60 tadpoles). The two field settings consisted of mesh enclosures (200 L, 35 tadpoles) placed in two different natural ponds. We also included a non-experimental sample used to evaluate the realism of the experimental venues: wild tadpoles collected in the source pond from which the experimental tadpoles originated and in the two ponds used in the field enclosure venue.

The impact of setting may vary among venues. In laboratory and mesocosm venues, setting corresponds to differences in volume and surface area, both of which are known to influence the performance of aquatic organisms (Pearman 1993; Petersen *et al.* 1999; Spivak, Vanni & Mette 2011). In the field enclosure venue, setting reflects a difference between ponds that are shaded to different degrees, and this in turn can affect numerous traits of amphibian larvae (Skelly, Freidenburg & Kiesecker 2002; Schiesari 2006; Van Buskirk 2011). Thus, settings are not directly comparable across venues, and should be interpreted as representing different venue types that often occur in ecological experimentation. This can be viewed as a strength of the design, because repeating the experiment in multiple settings improves the generality of our conclusions.

We designed protocols to reflect methods currently used by experimentalists studying amphibians in laboratory, mesocosm, and field experiments (e.g., Skelly 2002; Fraker *et al.* 2009). Laboratory bins contained aged tap water in a room maintained at 20 °C on a 12:12 light:dark cycle. Three times per week we siphoned out half the water and replaced it; on two occasions we renewed all the water in the bins. Two types of water were used to replenish the bins, and this allowed us to manipulate apparent predation risk. Half the bins received water from an 80 L tub in which two *Anax imperator* dragonfly larvae had each been fed 300 mg of *Rana temporaria* tadpoles, three times per week. These bins therefore contained waterborne dragonfly kairomones and tadpole alarm chemicals, which are frequently used to simulate

predation risk in laboratory experiments (Kraft, Franklin & Blows 2006; Urban 2008; Fraker *et al.* 2009). Bins in the no-predator treatment received aged tap water. Tadpoles were fed three times per week with a 4:1 ratio of finely-ground rabbit food and Tetramin fish flakes. The quantity of food was adjusted continuously so that tadpoles received approximately 20% of their mass per day.

The mesocosms were arranged in a field at the University of Zurich, Switzerland, filled with tap water 27 days before the experiment began, and kept covered with lids constructed of 43% shade cloth. We stocked each mesocosm with dried leaf litter (40 g in the 80 L setting and 400 g in the 675 L setting) and rabbit chow (2 g in 80 L tubs and 10 g in 675 L tanks), and made several additions of water and zooplankton collected from a nearby pond. No supplemental food was added during the experiment. Non-lethal predation risk was manipulated by placing floating cages containing an *A. imperator* larva within half the mesocosms (1 cage in the 80 L setting, 2 cages in the 675 L setting). The cages were about 1 L in volume, constructed of an 11 cm length of plastic tubing with window screen on the ends. We fed each predator 300 mg of tadpoles three times per week; this produced a concentration of waterborne kairomones in the 80 L mesocosms identical to that in the laboratory venue (Table 1). Predator-free mesocosms contained empty cages.

The field enclosure venue consisted of two sets of six enclosures in each of two different ponds, chosen to represent typical conditions under which field experiments on larval amphibians are conducted. One pond, here called the “sunny” pond, has a muddy substrate with 87% coverage by submerged aquatic vegetation and a canopy cover of 9.5%, measured as the obstructed sun arc between 10:15 and 17:00 local time in late April. The sunny pond is 13.7 km N of Zurich (47.49086 N, 8.53638 E). The second pond, called the “shady” pond, is 3 km S of the sunny pond (47.46484 N, 8.53542 E) and has a substrate of decomposing leaf litter, 15% coverage by emergent aquatic vegetation, and 60% canopy cover. Both ponds contained numerous predators, including adult *Notonecta glauca* backswimmers, larval *Aeshna cyanea* and *A. imperator* dragonflies, and larval *Dytiscus marginalis* beetles. The enclosures had a surface area of 1 m^2 and were constructed of fiberglass window screen (0.5 mm mesh) covering a wooden frame, reinforced with hardware cloth on the bottom and covered with a lid of 43% shade cloth. The substrate was a mixture of leaf litter, vegetation, and mud that we scooped from 1 m^2 of the pond immediately adjacent to the enclosure and searched to remove any potential predators.

We set the enclosures initially at a depth of about 30 cm, but the water level declined during the experiment so that the average volume was 200 L. The caged-predator treatment contained a single caged *A. imperator* larva, which was fed 300 mg *R. temporaria* tadpoles three times per week. The no-predator enclosures contained an empty cage. All cages were rotated among pools or enclosures within treatments on each feeding event to even out potential differences among individual dragonflies.

Experimental units were arranged in spatial blocks in all six settings, and treatments were assigned at random within blocks. Replication and other experimental details are summarized in Table 1.

The experimental animals came from seven clutches of *R. temporaria* eggs collected from a pond 1.3 km SSE of the sunny pond (47.48103 N, 8.54500 E). We initiated the experiment on 6 April 2009, when the tadpoles were two days old (stage 23; Gosner 1960). Each clutch contributed an equal number of individuals to every experimental unit as far as possible. For example, each of the field enclosures received 5 tadpoles from every clutch, and the 675 L mesocosms received 8 tadpoles from each of three clutches and 9 from the remaining four clutches. In the 1 L laboratory setting, four clutches contributed one replicate each and three clutches contributed two replicates. Tadpoles remained in their assigned bins, tubs, or tanks until they reached stage 42 (forelimb emergence), with the exception of those in field enclosures. In that venue, we transferred all tadpoles to 80 L mesocosms on campus just after the first individuals reached metamorphosis, because we were unable to reliably collect metamorphs in the complex substrate of the enclosures. The impact on the results of bringing tadpoles to campus was probably minor, because the average individual spent only 4.3 d in the mesocosms before metamorphosing (6% of the larval period). The experiment continued, and metamorphs were collected daily, until all individuals reached stage 45.

MEASURING TRAITS

Life history traits. – We recorded body mass and developmental stage on 28 April, when tadpoles were 24 days old. This included all animals in the laboratory experiment, a subsample of those in the mesocosms (6 per 80 L tub, 8 per 675 L tank) and field venue (10 per enclosure), and samples of wild tadpoles taken from the experimental ponds (10 tadpoles per pond) and the

pond where egg clutches were originally collected (9 tadpoles). Wild tadpoles were collected by dip-netting for a few minutes in each of several parts of the pond, and immediately returning individuals with no visible tail damage to the lab for measurement. We weighed all tadpoles and determined developmental stages from photographs of each individual. Metamorphic performance was represented by survival, mass, and age at stage 45.

Behavior. – We observed behavior in the laboratory and mesocosm venues on 22 April, when tadpoles were 18 days old. Each experimental unit was visited repeatedly, and the number of visible animals that were active (swimming or feeding) and inactive (resting) was counted. The 675 L tanks were visited 6 times during the day, the 80 L tubs 11 times, and both 1 L and 5 L laboratory bins 19 times. Tadpoles not visible to the investigator in the mesocosms were recorded as hiding in the leaf litter. We estimated the number alive in each mesocosm on 22 April assuming constant per capita daily mortality. There were two alternative measures of activity in mesocosms. If we assumed that hiding animals were inactive, activity was the number active divided by the estimated number alive, which probably underestimates true activity. The other measure of activity, the number active divided by the number observed, effectively assumed that hiding and visible tadpoles were equally active, which overestimates activity. Behavioral data were not collected in the field enclosures because tadpoles could not be seen.

Morphology. – We used the photographs from 28 April to measure morphological shape of all tadpoles in the laboratory experiment, 10 tadpoles per enclosure in the field venue, 6 tadpoles per 80 L mesocosm, 8 tadpoles per 675 L mesocosm, and a total of 29 wild tadpoles. Each image had lateral and ventral views of the tadpole in a water-filled Plexiglas chamber. Animals were returned to their experimental unit or pond after photography.

We used geometric morphometric analyses to describe variation in tadpole shape. Geometric methods correct for differences in size, location, and orientation between specimens, and use the relative positions of landmarks to quantify shape (Zelditch *et al.* 2004). We used the image analysis program ImageJ to place 22 side-view landmarks and 13 bottom-view landmarks on each photograph (defined in Van Buskirk 2011). Specimens were scaled to unit size and rotated to a common orientation using Procrustes superimposition. We then projected the landmarks back into Euclidean space and subjected them to Principal Components Analysis, and retained the most important components (termed relative warps, RWs) to describe variation in shape.

The first four RWs were included for the lateral view, comprising 83.4% of all shape variation; three RWs were included for the ventral view, comprising 85.5% of variation. Illustrations of the RWs generated by a thin plate spline algorithm are in Supporting Information A. Lateral and ventral RWs were in some cases correlated with each other. For example, lateral RW1 was positively correlated with ventral RW1 (r = 0.80, N = 350 tadpoles), because both RWs represent a short tail and large head/body. Ventral RW1 was also correlated with lateral RW2 (r = -0.49), reflecting an association between a wide and deep head/body, especially in the gut region, and a deep anterior part of the tail.

ANALYSES

Analyses of variance evaluated the influence of venue, setting nested within venue, predator treatment, and their interactions on tadpole phenotypes. We began with multivariate analyses for performance traits (body mass and developmental stage at 24 days, survival to metamorphosis, mass and age at metamorphosis) and morphological shape (the 7 RWs). Masses were log transformed before analysis, and survival and behavioral traits were arcsine square root transformed. Supporting Information D reports results of a parallel set of analyses on a set of size-corrected length measures, included for comparison with earlier studies of amphibian morphology.

To evaluate the precision of estimated trait values in each of the settings, we calculated average coefficients of variation (CV) among replicates for life history and behavior, and variance among replicates was measured for shape components. The CV is undefined for relative warps, which have an average value of zero.

Results

Life history. – Venue and setting strongly influenced the five life history traits in a multivariate analysis of variance (venue: Wilks' $F_{10,44}$ = 70.9, P <0.0001; setting nested within venue: Wilks' $F_{15,61}$ = 3.48, P = 0.0003). Univariate tests revealed that venue had significant effects on all traits, whereas setting was important only for age and size at metamorphosis (Table 2). Tadpoles raised under laboratory conditions developed fastest and were frequently the heaviest (Fig. 1). The

field venue led to decreases in all fitness related traits: tadpoles developed slowly and metamorphosed at small sizes in the enclosures. Survival was lowest in the field enclosures, for unknown reasons (shady pond: 0.650; sunny pond: 0.837), and approximately equal in the laboratory and mesocosm venues (ranging from 0.90 to 0.97). Wild tadpoles from the sunny and shady ponds were similar in size and developmental stage to experimental tadpoles raised in the same pond (Fig. 1A, B). Tadpoles from the source pond were larger and more developmentally advanced than those in the other two ponds.

There was no effect of the caged-dragonfly treatment on life history (MANOVA; Wilks' $F_{5,22}$ = 1.76, P = 0.1631), but Fig. 1 and the univariate analyses in Table 2 suggest that predators caused somewhat reduced early growth and delayed development at both tadpole and metamorph stages. More important was the highly significant treatment-by-venue interaction (Wilks' $F_{10,44}$ = 4.82, P = 0.0001). Predation risk induced larger body size at metamorphosis in the laboratory, but slightly smaller size in the field (Fig. 1C). Metamorphosis was delayed by at least a week in mesocosms when predators were present, but was if anything accelerated by predation risk in the laboratory (Fig. 1D). Interactions between treatment and setting (nested within venue) for body size arose because tadpoles in the 1L lab bins grew especially large under predation risk, and tadpoles in the 80L mesocosms were especially small with predators (Fig. 1A, C).

Behavior. – Activity was mostly influenced by treatment and to a lesser extent by venue (Fig. 2, Table 2). The comparison between mesocosm and laboratory venues depended on assumptions about the behavior of invisible animals in mesocosms. If hiding animals were ignored, effectively assuming that they behave the same as those that were visible, then activity was greatly reduced in the lab in the absence of predators (Fig. 2A). On the other hand, if we assume that hiding animals were not moving, then activity was higher in the lab under predation risk (Fig. 2B). The dragonfly treatment caused somewhat lower activity in both venues, but the significant treatment-by-venue interaction indicated that the response in the lab was comparatively weak. In mesocosms, tadpoles exposed to predators reduced their activity by at least 70%, and in the 80 L setting fully 100% of individuals were either resting or hiding. Supporting Information B shows results for the proportion of individuals feeding, swimming, and hiding in the litter.

Morphology. – Morphological shape was highly sensitive to venue and setting (MANOVA on seven relative warps; venue: Wilks' $F_{14,40}$ = 51.5, P <0.0001; setting: Wilks' $F_{21,58}$ = 3.97, P =

0.0001). Laboratory tadpoles had a relatively long and shallow tail with a short and narrow head/body and reduced gut mass (lateral RW1 and ventral RW1; Fig. 3, Table 2). On most measures, animals in mesocosms were similar to those in field enclosures and the wild, although they were intermediate on lateral RW1 (Supporting Information C).

The caged-predator treatment induced numerous changes in shape (Wilks' $F_{7,20}$ = 20.5, P <0.0001), especially in the depth of the tail, attachment of the dorsal fin, relative gut mass, and orientation of the mouth and eyes (lateral RW3 and RW4; Fig. 3 and Supporting Information C). These responses were highly variable among venues (treatment-by-venue interaction: Wilks' $F_{14,40}$ = 11.1, P <0.0001). In many cases, tadpoles in the lab venue responded more weakly or in the opposite direction to predators than did those outdoors. This was true for lateral RW1, RW3, and RW4: the increasing arch and depth of the tail induced by dragonflies was absent in laboratory tadpoles (Fig. 3). But in other cases, it was the animals in mesocosms that showed a different, and usually greater, response to predators than those in the lab or field. Examples include lateral RW4 and ventral RW2: when exposed to caged predators, mesocosm tadpoles developed a deeper tail, shorter head/body, and narrower gut than did other tadpoles (Fig. 3, Supporting Information C).

Analyses of conventional morphometric lengths generally confirm the results shown here (Supporting Information D).

Precision. – Variation among replicates was not lower in the more controlled venues (Fig. 4). The 1 L laboratory setting had high variance for all types of traits because replicate observations were individuals rather than averages of multiple tadpoles. Other venues and settings had roughly equivalent precision. CV was higher for behavior than for other traits, and did not differ consistently among treatments. Samples from the wild showed relatively low precision.

Discussion

We compared three commonly used experimental venues to evaluate how outcomes depend on venue, and to shed light on constraints and trade-offs associated with choosing a venue. In agreement with previous work, our data show that venue can strongly impact results (Skelly &

Kiesecker 2001; Skelly 2002; Bell *et al.* 2003; Brown *et al.* 2006; Romanuk, Vogt & Kolasa 2009; but see Weigensberg & Roff 1996; Blaustein *et al.* 2004; Bancroft *et al.* 2007). We presented two kinds of findings in this study. First, there were strong effects of venue for nearly every trait we measured in developing amphibian larvae, including those related to individual fitness (stage and mass of tadpoles and metamorphs, and survival) and those with a weak or context-dependent connection to fitness (behavior and morphological shape). The venue effect was more pronounced in some traits, such as lateral RW1 and ventral RW1, than in others. The second kind of result was that phenotypic plasticity induced by predation risk depended on venue. Tadpoles in mesocosms exhibited greater behavioral and morphological plasticity, along with larger reductions in performance due to dragonflies, than those in the lab.

It is important to identify the differences among venues that contribute to variation in phenotype and performance, because these might be controlled or incorporated into future experimental designs. For example, variation among venues in development rate was probably related to temperature; tadpoles raised in the laboratory experienced relatively stable and warm temperatures, and therefore developed rapidly. Large body size at emergence in the laboratory, and to a lesser degree in mesocosms, may have been caused by more abundant food of higher quality than was available in field enclosures. Introducing lower-quality food and a diurnally fluctuating temperature regime might create more realistic performance in the lab, although these conditions may conflict with other experimental objectives.

Mesocosms are not natural (Jaeger & Walls 1989; Boone & James 2005), and it has been argued that their unnatural features generate unrealistic experimental outcomes (Carpenter 1996; Skelly 2002). Skelly and Kiesecker (2001; Skelly 2002) have used meta-analyses and experiments to suggest that amphibian growth and development rates in mesocosms are exceptionally high. But data on performance are problematic for appraising realism of experimental venues, because natural ponds are themselves quite variable. Even our limited sample of three natural ponds encompassed nearly the entire range of variation in growth and development observed across the three venues. Depending on which of the ponds is taken as a reference, we could use Fig. 1 to argue that either field enclosures, mesocosms, or even the laboratory is most realistic. But these differences in performance traits among venues may reflect not realism, but rather variation in temperature or resources that could just as well occur in nature. This argument applies less strongly to morphological shape, which is probably less sensitive to temperature and

resources. In this study, tadpoles in the mesocosm settings were morphologically similar to those in field enclosures and natural ponds. This suggests that mesocosms are acceptably realistic for at least morphological traits. Lab-reared tadpoles, on the other hand, were highly divergent in morphology from field and mesocosm specimens.

Our second main finding was that venues differed in the magnitude, and sometimes even the direction, of predator-induced plasticity. In this case, our data do not indicate which venue most closely mirrors nature. However, other studies comparing mesocosm estimates of plasticity with tadpole morphology in natural wetlands having both temporal and spatial variation in predator density indicate that the phenotypic reaction in mesocosms parallels that observed in nature (Van Buskirk & McCollum 1999; Van Buskirk & Schmidt 2000; Van Buskirk 2009). This suggests that, although mesocosms produced relatively high estimates of plasticity in our study, they at least accurately reflect the direction of response seen in nature.

Of the many factors that could cause differences in plasticity among venues and settings, two that seem especially likely are kairomone concentrations and the size of the experimental populations. Within the mesocosm venue, several traits responded more strongly to predators in the 80 L setting, which had higher kairomone concentrations and smaller numbers of tadpoles. Anti-predator reactions are known to scale with kairomone level (Van Buskirk & Arioli 2002; Schoeppner & Relyea 2008), and group size independent of density can modify behavior by affecting individual risk and the perception of risk (Elgar 1989; Van Buskirk *et al.* 2011). In field enclosures, the open mesh walls may have prevented us from successfully manipulating kairomone concentration and apparent predation risk. On the one hand, dilution of kairomones by water flow through the walls could reduce the difference between treatments. Alternatively, all the enclosure animals, including those in the predator-free treatment, may have been exposed to kairomones washed in from nearby wild predators (Chalcraft *et al.* 2005). Indeed, some life history and morphological traits measured in enclosures were most similar to those observed in mesocosms with caged dragonflies (e.g., mass and stage at 24 days, lateral RW1, and ventral RW2), suggesting that kairomone levels may have been high even in the predator-free treatment.

The conclusion that plasticity depends on kairomone concentration was not consistent with results from the laboratory venue. Kairomone concentrations in the lab were identical to those

in 80 L mesocosms, yet laboratory animals showed limited plasticity. It is unlikely that additional visual or tactile cues, not present in the lab, amplified the response of animals in mesocosms. Water-borne chemicals induce strong phenotypic plasticity in amphibian larvae (LaFiandra & Babbitt 2004; Kraft *et al.* 2006; Hettyey *et al.* 2010), and adding tactile or visual cues causes no additional reaction, at least for behavior (Stauffer & Semlitsch 1993; Kiesecker, Chivers & Blaustein 1996; Hickman, Stone & Mathis 2004; Jowers *et al.* 2006; Saidapur *et al.* 2009). We suspect instead that unknown features of the laboratory environment bias the expression of behavior and morphology. Identifying precisely which features are important would require further experiments.

This study confirms Skelly and Kiesecker's (2001) conclusion that precision is not greatly enhanced in the lab, contrary to conventional thinking (e.g., Lawton 1995). The 5 L laboratory bins containing groups of five tadpoles showed precision better than that in field enclosures for only one of the two ponds (Fig. 4A), and comparable to that in mesocosms. This result is particularly relevant for the choice of venue because precision is held among the key benefits favoring laboratory work (Lawton 1995; Morin 1998). Our results highlight instead a contrast between experimental setups where units contain small numbers of individuals, leading to relatively high variance among replicates, or large numbers of individuals. More replication is needed when few organisms are present within each replicate.

Our results could be used to defend any of the three venues. For example, the relatively realistic phenotypes exhibited in mesocosms, in combination with the low precision found in small laboratory bins, argues in favor of more natural experimental settings containing groups of individuals. For most traits, mesocosm experiments apparently yield high realism without sacrificing precision. This conclusion is not affected by the relatively distinct settings occurring in the field venue (different ponds) compared to the mesocosm venue (different sized containers). Precision was calculated as the coefficient of variation among replicates within settings, and realism was judged by comparing experimental with wild tadpoles. Both comparisons are statistically and conceptually identical for all venues and settings, even as the precise meaning of setting changes across venues. Some might conclude that higher phenotypic plasticity in mesocosms indicates that animals overestimate differences in predation risk in that venue. For others, the high variability among enclosures observed within one of the two ponds might argue for using lab or mesocosm venues, especially given other issues with field experiments such as

limits on replication and difficulty controlling certain conditions. Overall, the results confirm an age-old recommendation in ecology that the venue must be matched, one study at a time, to the prevailing constraints and the question at hand (Diamond 1986; Wilbur 1989; Morin 1998). Our data will help resolve these trade-offs in specific cases by clarifying the consequences for individual phenotypes.

Acknowledgements

Thanks to Mathias Fischer, Morana Mihaljević, Lorenzo Tanadini, and Iris Wurmitzer for help with the experiment, to the Zürich Veterinary and Nature Conservation offices for permits, and to the Swiss National Science Foundation for funding.

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Table 1. Summary of the experimental venues and settings. The concentration of kairomones in the caged-predator treatment is reported in two ways: the mass of live tadpoles consumed per volume and per week, and the density of dragonflies per volume. For wild tadpoles, the table lists the area and volume of the three ponds at maximum depth, and the number of tadpoles sampled from each pond.

Venue	Setting	No. of replicates	Surface area (m^2)	Volume (L)	No. of tadpoles	No. of caged *Anax*	Kairomone concentration	
							mg tad/L/week	*Anax*/L
Laboratory	Small bin	10	0.0228	1.1	1	--	11.25	0.0125
Laboratory	Large bin	6	0.06	5.2	5	--	11.25	0.0125
Mesocosm	Small tub	5	0.28	80	12	1	11.25	0.0125
Mesocosm	Large tank	4	1.35	675	60	2	2.67	0.0030
Field enclosure	Sunny pond	3	1.0	200	35	1	4.50	0.0050
Field enclosure	Shady pond	3	1.0	200	35	1	4.50	0.0050
Wild tadpoles	Source pond	1	2500	6.2×10^5	9	--	--	--
Wild tadpoles	Sunny pond	1	650	2.0×10^5	10	--	--	--
Wild tadpoles	Shady pond	1	300	1.0×10^5	10	--	--	--

Table 2. Results of univariate analyses of variance on life history, behavior, and morphological shape in lateral and ventral view. Entries are the *F*-value (above) and *P*-value (below). Numerator df are listed at the top, and the denominator for all effects was the interaction between treatment and block nested within setting and venue (df = 26). For activity, df were reduced because there were only two venues (lab and mesocosm). Bold text highlights tests significant at α = 0.05. Masses are log-transformed, survival and behavioral traits are arcsin-sqrt transformed, and shape components are relative warps derived from geometric morphometric analyses.

	Source of variation				
Response variable	Treatment × Treatment Setting(Venue) (df=1)	Venue (df=2)	Setting(Venue) (df=3)	Treatment × Venue (df=2)	(df=3)
Life history traits					
Tadpole mass at age 24 days	**5.55** **0.0263**	**188.01** **<0.0001**	1.30 0.2961	**16.53** **<0.0001**	**4.62** **0.0101**
Developmental stage at age 24 days	**5.10** **0.0326**	**113.49** **<0.0001**	1.90 0.1542	**9.28** **0.0009**	2.61 0.0731
Survival to metamorphosis	0.00 0.9772	**47.89** **<0.0001**	2.69 0.0672	0.06 0.9393	0.47 0.7041
Mass at metamorphosis	2.56 0.1214	**125.28** **<0.0001**	**3.39** **0.0330**	**8.63** **0.0013**	**4.71** **0.0094**
Age at metamorphosis	**8.95** **0.0060**	**264.92** **<0.0001**	**3.78** **0.0225**	**9.58** **0.0008**	0.45 0.7192
Behavior at age 18 days					
Activity (hiding not counted)	**84.13** **<0.0001**	**6.14** **0.0234**	**4.99** **0.0189**	**56.73** **<0.0001**	**10.04** **0.0012**
Activity (hiding are inactive)	**40.15** **<0.0001**	**23.49** **0.0001**	**3.82** **0.0415**	**21.04** **0.0002**	2.50 0.1105

Table 2 continued.

	Source of variation				
Response variable	Treatment × Treatment Setting(Venue) (df=1)	Venue (df=2)	Setting(Venue) (df=3)	Treatment × Venue (df=2)	(df=3)
Morphological shape components at age 24 days					
lateral RW1	1.01 0.3237	**263.13** **<0.0001**	**6.32** **0.0023**	**8.73** **0.0013**	0.60 0.6194
lateral RW2	2.53 0.1235	**8.14** **0.0018**	1.50 0.2387	1.35 0.2758	0.29 0.8339
lateral RW3	**12.76** **0.0014**	2.00 0.1551	2.97 0.0502	**9.45** **0.0008**	0.50 0.6867
lateral RW4	**39.64** **<0.0001**	**4.13** **0.0276**	**7.31** **0.0010**	**14.68** **<0.0001**	1.35 0.2793
ventral RW1	0.04 0.8342	**216.20** **<0.0001**	0.22 0.8830	3.26 0.0545	0.69 0.5684
ventral RW2	**39.52** **<0.0001**	**18.76** **<0.0001**	**5.58** **0.0043**	**51.74** **<0.0001**	**8.22** **0.0005**
ventral RW3	**7.87** **0.0094**	**27.00** **<0.0001**	2.80 0.0601	**12.80** **0.0001**	0.10 0.9588

Figure 1. Life history responses of *Rana temporaria* tadpoles to manipulation of venue and non-lethal predation risk (treatment). Developmental stage is defined in Gosner (1960). Metamorphic mass and age were measured at stage 45, after the tail was fully resorbed. Symbols represent means ± 1 SE.

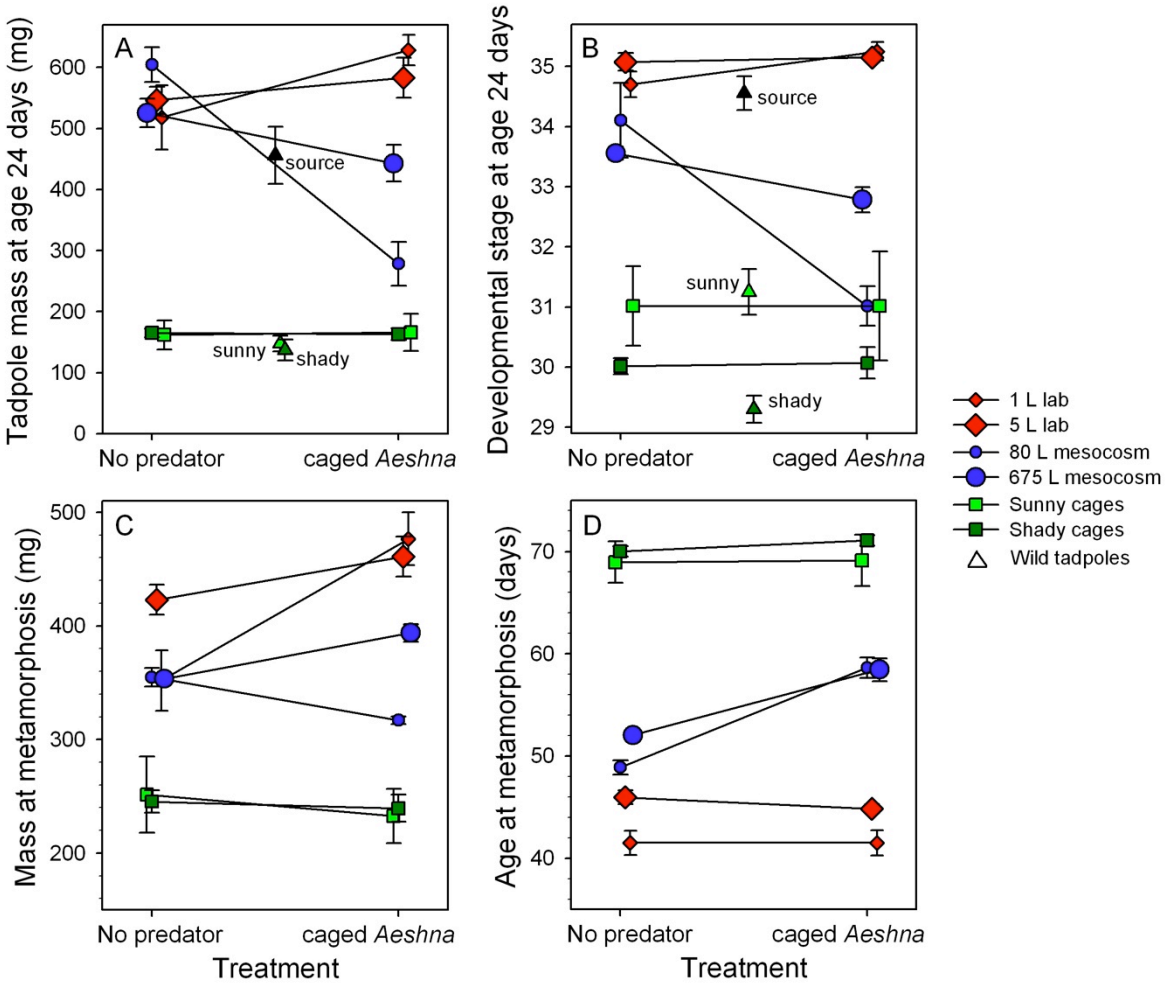


Figure 2. *Rana temporaria* tadpole activity measured at age 18 days. Symbols represent means ± 1 SE of the proportion of visible tadpoles that were active (A) or the proportion of tadpoles active under the assumption that hiding individuals were inactive (B).

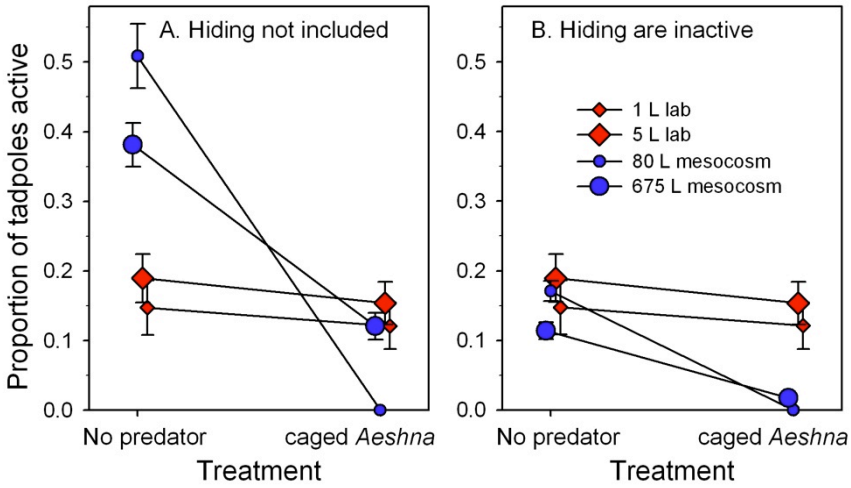


Figure 3. Morphological shape components measured at 24 days of age. Tadpole drawings illustrate shape changes represented by relative warps: the gray/black outlines show tadpoles with scores 2 SD above/below the mean form (lateral RW1 and ventral RW1), 3 SD above/below the mean (lateral RW3), or 4 SD above/below the mean (ventral RW2). Error bars indicate ± 1 SE based on replicate tubs (experiment) or individual tadpoles (wild samples).

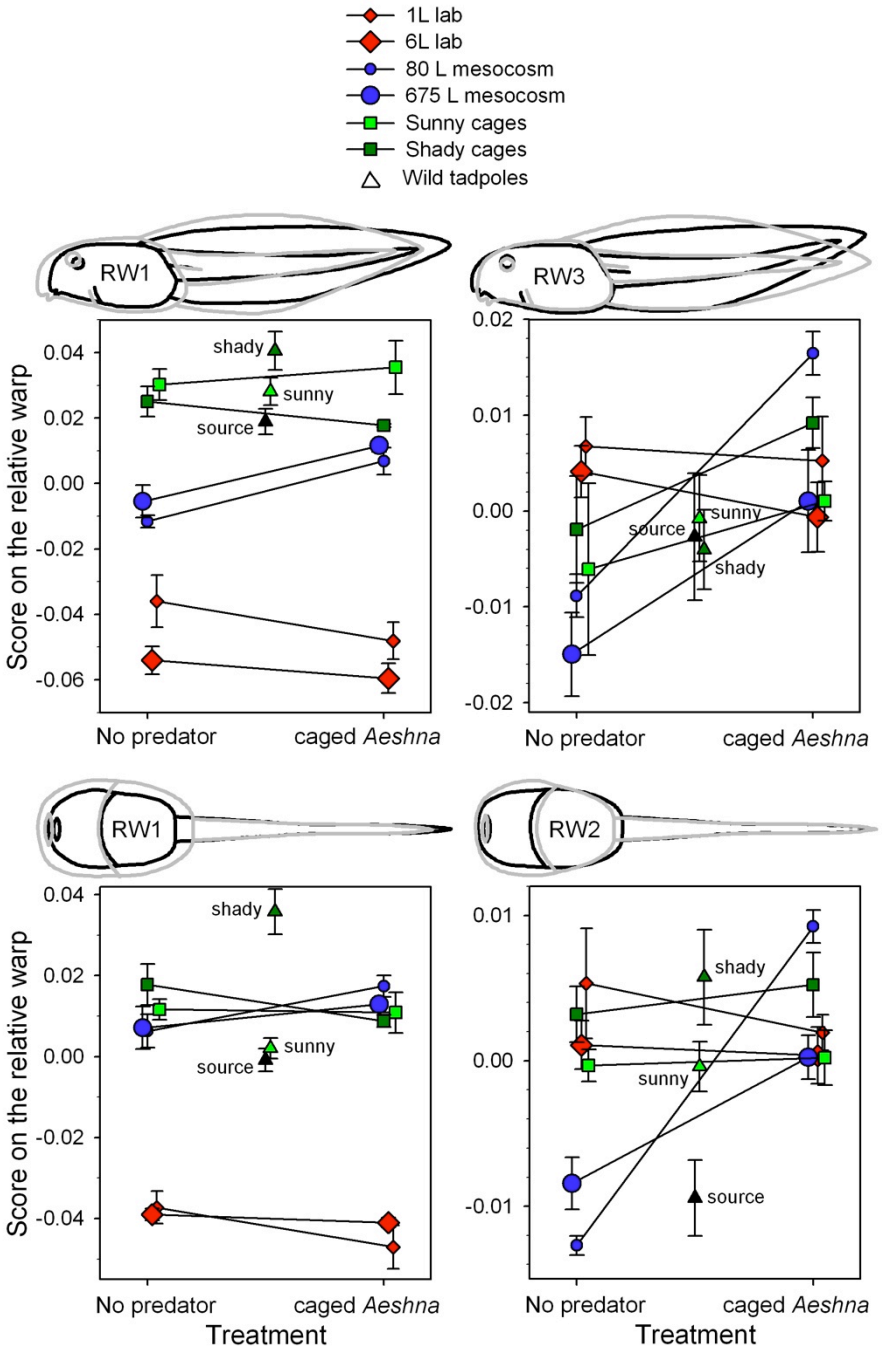
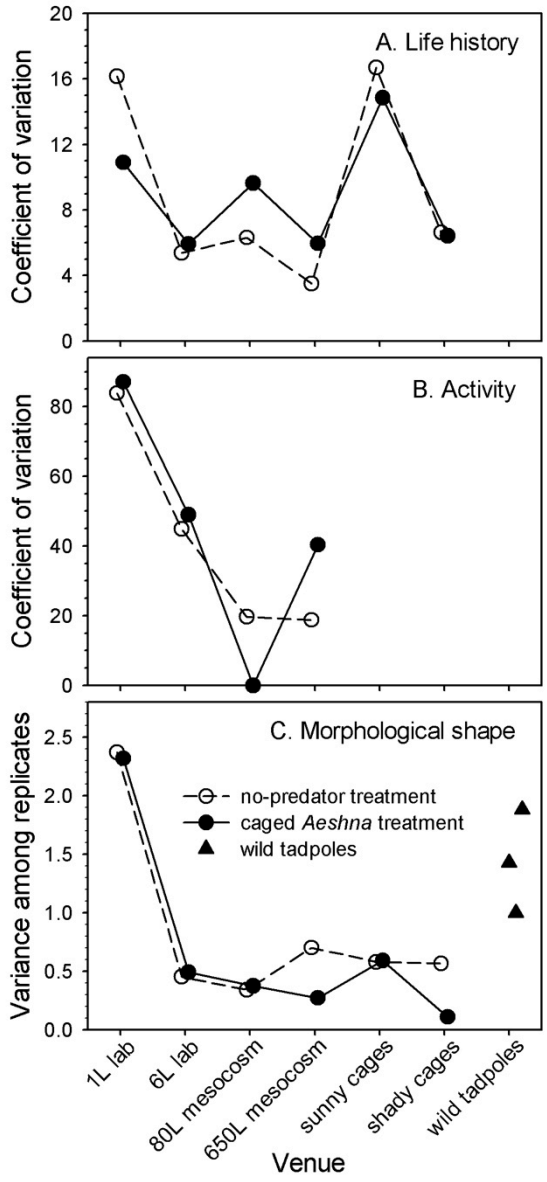
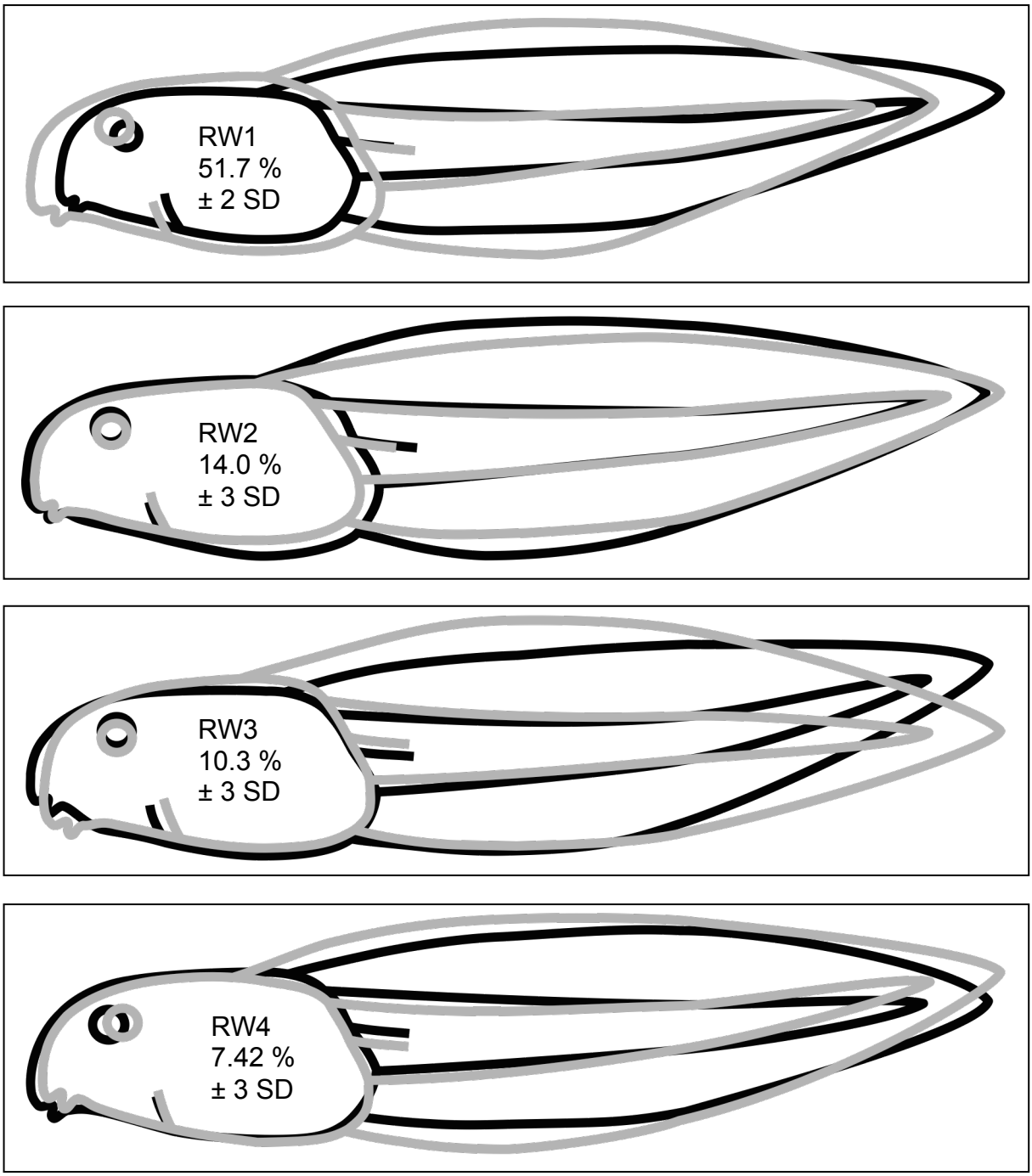


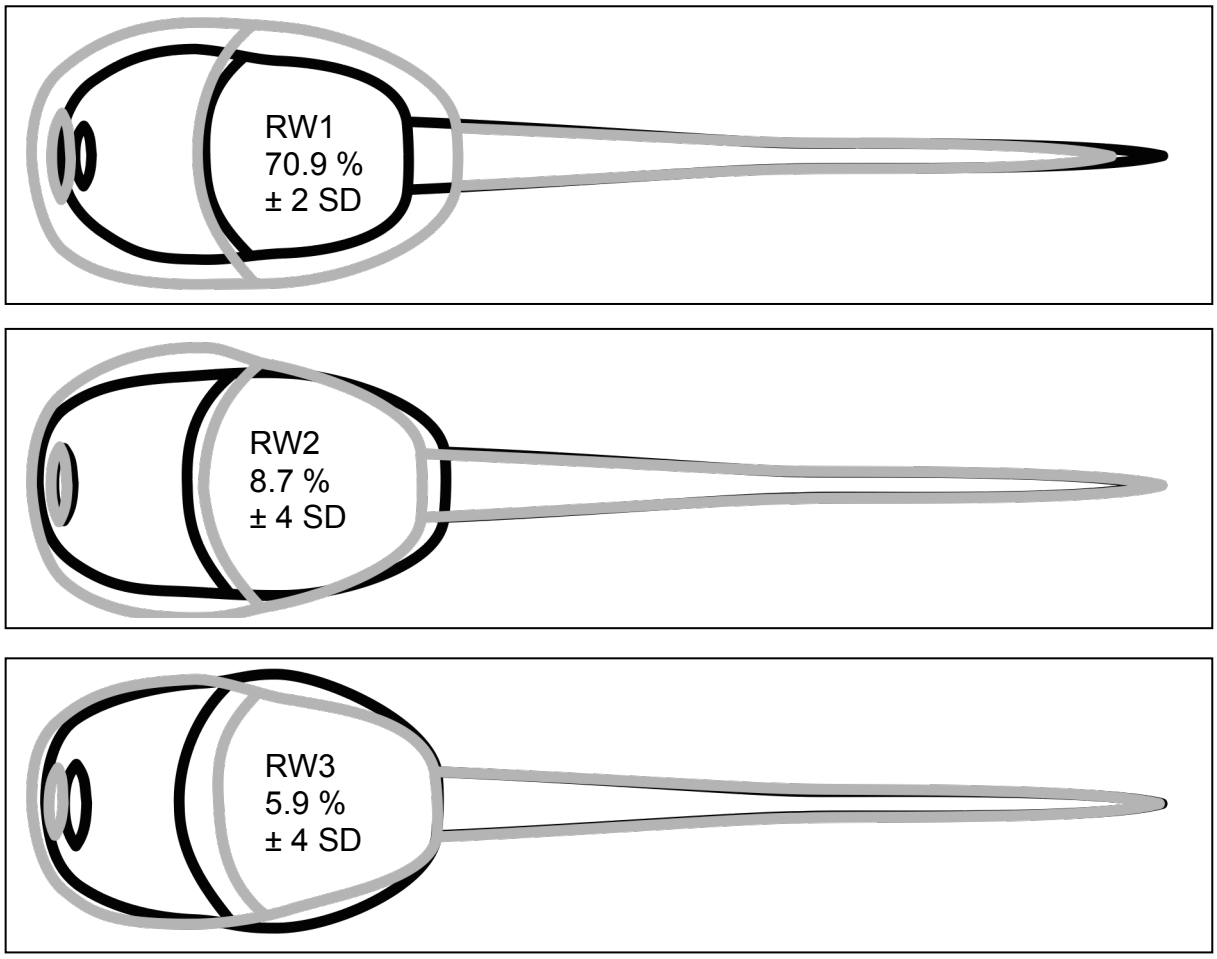
Figure 4. Precision of the estimate of tadpole life history, behavior, and morphology in each venue. Points show the average coefficient of variation (A, B) or variance (C) among replicates. The numbers of traits included are 5 in A (Fig. 1 and survival), 2 in B (Fig. 2), and 7 in C (lateral RW1-4 and ventral RW1-3). Variance components for morphological traits were multiplied by 104. The sequence of venues along the horizontal axis is approximately in decreasing order of experimental control and increasing order of ecological complexity. Low values of variation among replicates indicate high precision.



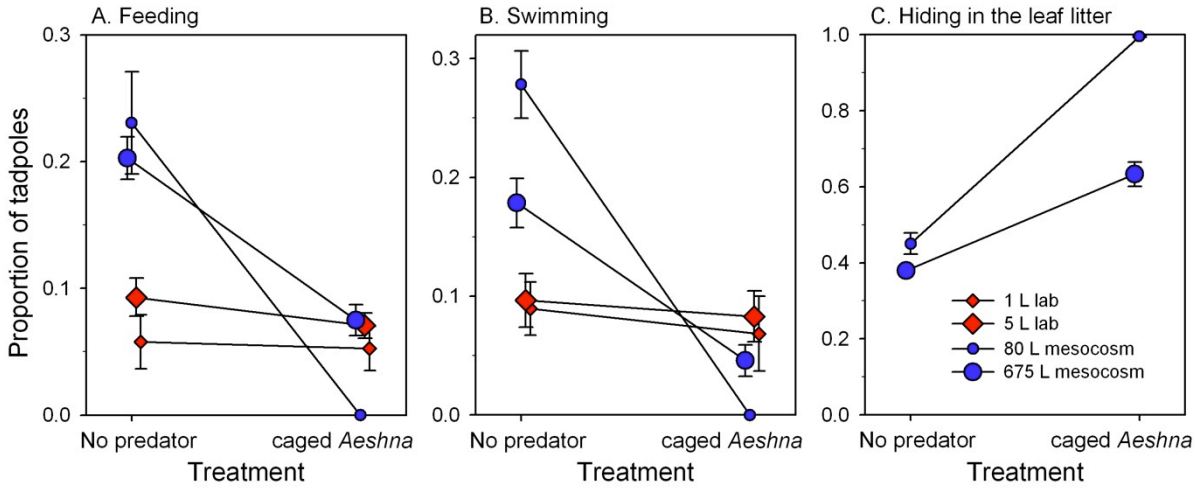
Supporting Information A. Relative warps representing *Rana temporaria* tadpole shape, calculated from geometric morphometric analysis of 22 landmarks on the lateral view and 13 landmarks on the ventral view. Percent of total shape variation explained by each relative warp is labeled on the figure. Gray outlines depict individuals with scores on the relative warp above the average by the number of standard deviation units indicated on the figure; black outlines represent individuals with scores below the average by an equal amount.



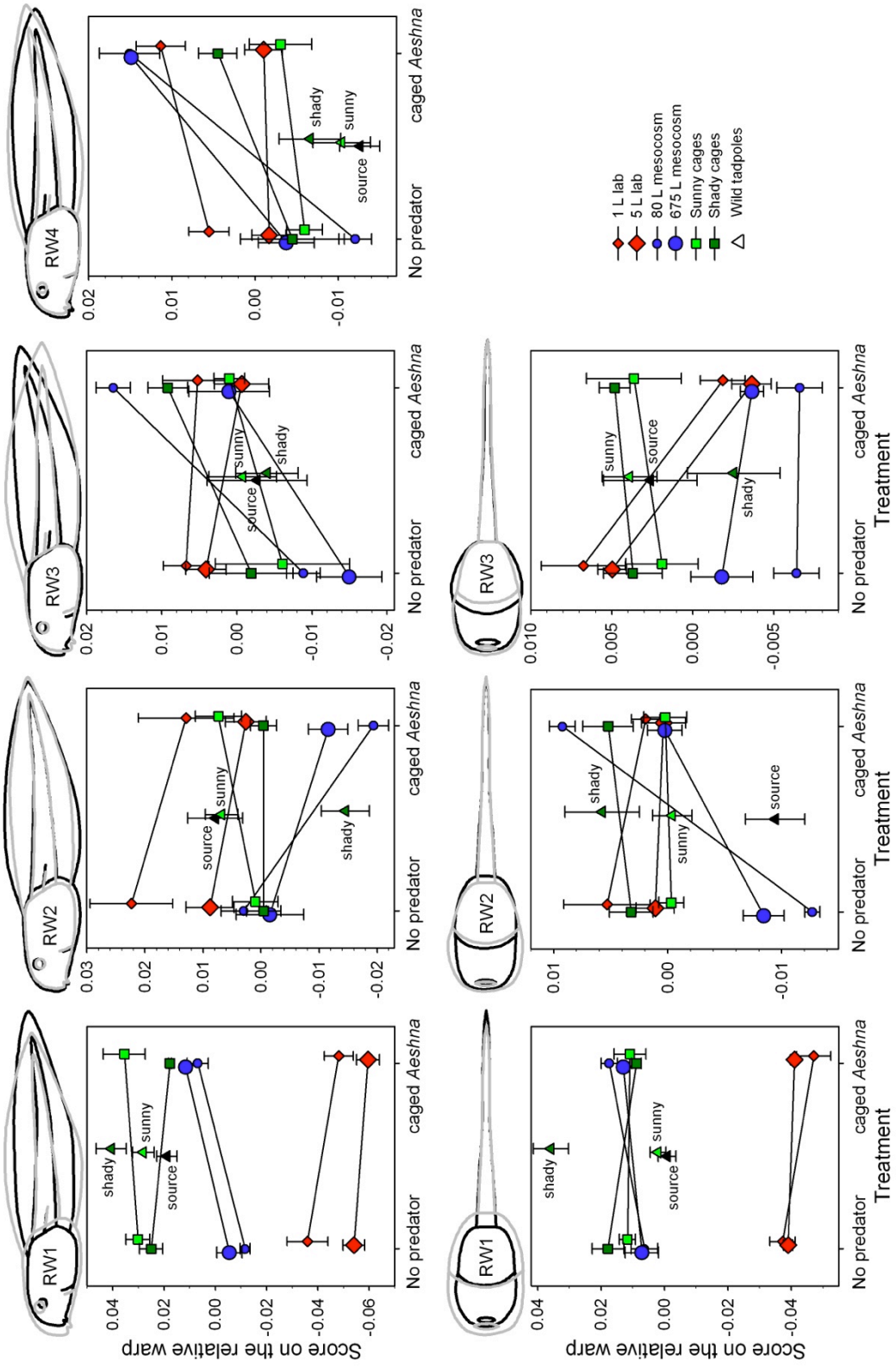
Supporting Information A continued.



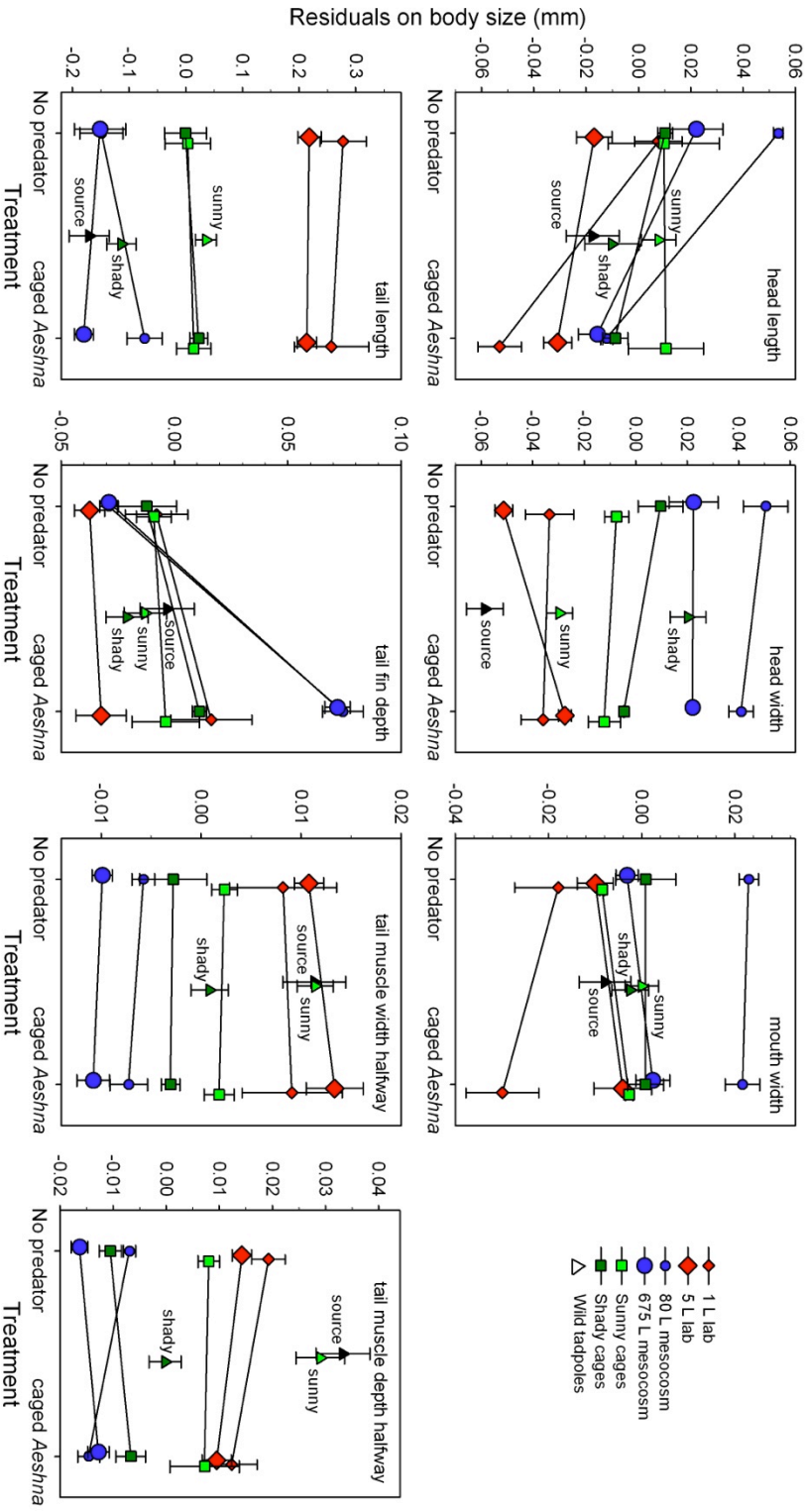
Supporting Information B. The proportion of visible *Rana temporaria* tadpoles that were feeding (A) and swimming (B), and the proportion of all tadpoles that were not visible above the litter in outdoor mesocosms (C). Behavior was observed at age 18 days. Symbols represent means ± 1 SE.



Supporting Information C. Morphological shape components measured at 24 days of age. Tadpole drawings illustrate shape changes represented by relative warps: the gray/black outlines show tadpoles with scores 2 SD above/below the mean form (lateral RW1 and RW2, and ventral RW1), 3 SD above/below the mean (lateral RW3 and RW4), or 4 SD above/below the mean (ventral RW2 and RW3). Error bars indicate ± 1 SE.



Supporting Information D. Morphology of tadpoles at age 24 days measured by conventional size-corrected lengths. The figure shows means ± 1 SE of residuals after regression against mass and mass^2. The table reports results of ANOVAs on size-corrected measures. Treatment was presence or absence of a caged *Anax* dragonfly larva; venue was laboratory, outdoor mesocosm, or field cage; setting was the size of the laboratory tub, volume of the mesocosm, or pond containing the field cages (Table 1). Numerator df are listed at the top of the table, and the denominator for all effects was the interaction between treatment and block nested within setting and venue (df = 29). The measurements are defined below the table.



Supporting Information D continued.

Response variable	Treatment (df=1)	Venue (df=2)	Setting(Venue) (df=3)	Treatment×Venue (df=2)
Relative head length	**31.95**	**18.10**	1.21	**3.91**
	0.0000	**0.0000**	0.3242	**0.0315**
Relative head depth	2.80	0.36	1.53	2.81
	0.1049	0.7013	0.2268	0.0769
Relative head width	0.14	**97.20**	**3.47**	2.06
	0.7111	**0.0000**	**0.0287**	0.1453
Relative tail length	0.17	**75.32**	0.99	0.25
	0.6851	**0.0000**	0.4119	0.7835
Relative tail fin depth	**92.33**	**27.60**	**13.62**	**42.54**
	0.0000	**0.0000**	**0.0000**	**0.0000**
Relative muscle width at halfway	0.00	**88.03**	**3.28**	0.59
	0.9851	**0.0000**	**0.0350**	0.5635
Relative muscle depth at halfway	1.09	**67.09**	**5.39**	0.99
	0.3055	**0.0000**	**0.0045**	0.3823
Relative mouth width	0.02	**17.84**	**6.79**	0.24
	0.8851	**0.0000**	**0.0013**	0.7920

The morphological traits are defined as follows (landmark numbers in Van Buskirk 2010): head length = tip of nose to point where ventral edge of tail muscle meets head/body (lateral-view landmark #1 to #14); head depth = upper edge to lower edge of the head/body at 2/3rds the distance from #1 to #14 (lateral #10 to #11); head width = left to right edge of the head/body at the widest point of the gut mass (ventral #6 to #7); tail length = point where the notochord (identified from the pattern of myotomes) meets the head/body to the tail tip (lateral #13 to #22); tail fin depth = dorsal edge of the tail fin at the deepest point to the ventral edge directly below that (lateral #16 to #17); tail muscle width at halfway = left to right edges of the tail muscle at half the distance between lateral #13 and #22 (ventral #11 to #12); tail muscle depth at halfway = upper to lower edges of the tail muscle at half the distance between lateral #13 and #22 (lateral #19 to #20); mouth width = left to right edges, defined as the points where anterior and posterior labial tooth rows converge (ventral #2 to #3).

THE EFFECTS OF ROAD SALT ON LARVAL LIFE HISTORY TRAITS AND BEHAVIOR IN *RANA TEMPORARIA*

Jasmin D. Winkler and Gina Forte

Amphibia-Reptilia (2011) 32, 527-532

Abstract. We investigated the effects of road salt on larval life history traits in a European amphibian species, the common frog *Rana temporaria*. Our main finding was that tadpoles showed decreased survival and activity at increased salinities, and that a sodium chloride control lacking the toxic substance ferrocyanide found in road salt had the same effect on tadpole survival as a road salt treatment at the same concentration of 2000 mg/l. Road salt caused increased mortality even at the smallest concentration tested, 500 mg/l (304 mg/l chloride). Activity of surviving tadpoles was only impacted at a higher salt concentration (2000 mg/l).

Introduction

The main road deicer used during winter in Europe and North America is sodium chloride, applied annually in thousands of tons to roads and highways to prevent traffic problems (Environment Canada, 2001). Eventually, road salts run off as solutes to the surroundings and the groundwater, leading to salt-contaminations of wetlands, streams and drinking water. Freshwater organisms are particularly sensitive to water salinization, and road salt application is associated with decreases in biomass and diversity of freshwater communities (Demers, 1992; Isabelle et al., 1987; Karraker et al., 2008; Richburg et al., 2001).

There are few measures of salt levels in European freshwater systems, but the concentrations may be quite high because large amounts of salt are applied to roads. For example, Switzerland uses 8-40 metric tons of road salt annually per lane-kilometer (ASTRA, Bundesamt für Strassen, www.astra.ch). In North America, where similarly large amounts of road salt are applied during winter (between 0.5-28.3 metric tons per lane-kilometer; National Research Council 1991), it is known that chloride concentrations can reach 4000 mg/l in freshwater wetlands, equaling a salt concentration of 6590 mg/l. Short term chloride concentrations measured in road run off may exceed 18'000 mg/l (Environment Canada, 2001). Although biological systems can take up low levels of sodium, sodium chloride tends to accumulate over time. In North America and Sweden, chloride concentrations in streams have been rising over the past decades and some wetlands show chronic salt contamination, causing long term effects of road salt application (Karraker, 2008; Kaushal et al., 2005; Thunqvist, 2004). Salt concentrations can also naturally be high in places that are neither close to the coast nor have high salt influx due to anthropological causes; a field study in Germany (Viertel, 1999) revealed salinities in a natural reserve as high as 4500 mg/l due to salty ground water.

Amphibians are generally very sensitive to osmotic stress, particularly during their aquatic larval stage (Ultsch et al., 1999); if salinity levels in the environment increase, osmoregulation may be disrupted, changing internal osmolality and eventually larval development and physiology (Gosner and Black, 1957; Shoemaker and Nagy, 1977; Ultsch et al., 1999). The majority of amphibian species studied to date show increased mortality, abnormal development, and reduced growth and activity in a saline environment (Turtle, 2000; Sanzo and Hecnar, 2006; Karraker, 2007; Karraker et al., 2008; Collins and Russell, 2009; Denoël et al., 2010; Squires et al.,

2010; Harless et al., 2011; also see review by Karraker, 2008). However, some species, and in some cases distinct populations, have evolved salt tolerance and are moderately euryhaline, including the natterjack toad (*Bufo calamita*), the European green toad (*Bufo viridis*) and the crab-eating frog (*Rana cancrivora*) (Gordon, 1962; Dunson, 1977; Gomez-Mestre and Tejedo, 2003).

To date, effects of sodium chloride have been studied in around 20 amphibian species, but only few species have been investigated for their tolerance to road salt, which includes compounds other than sodium chloride (e.g. Karraker et al., 2008; Petranka and Doyle, 2010). Road salts are often supplemented with the anti-caking agent ferrocyanide, a substance toxic particularly to aquatic organisms. Ferrocyanide has been reported to reduce survival in larval southern leopard frogs (*Rana sphenocephala*) and tadpoles of the boreal toad (*Bufo boreas*), with determined 96-h LC50 concentrations of 24.5 mg/l and 12.7 mg/l, respectively (Calfee and Little, 2003). A kilogram of commercial Swiss road salt contains 20 mg ferrocyanide, which could produce critical ferrocyanide levels at relatively low salt concentrations.

Given the intense application of road salt and the high road density in parts of northern Europe (e.g. road density in Switzerland is between 2.7 and 4 lane km per km^2 land (Schmidt and Zumbach, 2008), it is particularly pressing to investigate the tolerance of European amphibians to human induced salinization. The aim of this study was to investigate the effects of a commonly used deicing salt on survival and performance of the common frog (*Rana temporaria*) during the larval stage. We predict reduced survival and overall tadpole performance due to increased levels of salinity, and expect the effects of road salt to be different from those of pure sodium chloride. Our results are discussed in the context of observed salt concentrations in natural water bodies used as breeding ponds by *R. temporaria*.

Materials and Methods

Study species

R. temporaria, a widespread amphibian throughout much of Europe, is especially likely to be affected by human salt input. This species starts to breed in late winter or early spring, just when

wetlands are receiving run-off from melting snow, and breeding sites can be located immediately adjacent to roads (Meyer et al., 2009). To assess the effect to deicing salt on amphibian development, *R. temporaria* tadpoles were raised in outdoor mesocoms under different salt concentrations.

Experimental animals

R. temporaria tadpoles originated from crosses of wild caught adult frogs from a large breeding population in northern Switzerland (47°36'N, 8°40'E). The breeding pond is surrounded by forest (around 70%) and agriculturally used meadows (around 30%); the distance to the nearest road is around 530 m, and water salinity measured in spring 2011 was between 70 and 90 mg/l. Artificial crosses followed the protocol from Räsänen et al. (2003) with some modifications (also see Hangartner, 2010). In short, males were injected with fish hormone LHRH (H-7525, Bachem AG) 2 hours before crossing to stimulate sperm production (100 µl/10 g body mass of a 1 mg/100 ml sterile isotone saline solution), and placed individually in plastic boxes containing a small amount of 10 % Amphibian Ringer solution (Rugh, 1962). Eggs from each female were stripped and divided into individual containers, and covered with sperm solution from a single male to produce full sib families. Fertilized eggs were flooded with Ringer solution and kept outdoors until hatching, and all sires and dams were released. Nine days after hatching, on 6 April 2010, a total of 216 tadpoles (at Gosner 25 (Gosner, 1960)) from 6 different full-sib families, all sired from different parents, were chosen for the experiment. Each experimental unit received 2 tadpoles from each of the 6 full-sib families, i.e. a total of 12 tadpoles. Before tadpoles were released into the experimental units, they were first kept in temporary 1 l bins, where they were acclimated to the corresponding treatment by repeatedly adding small amounts of the appropriate water type to each bin over the course of a day. On 7 April, all tadpoles were released into their assigned mesocosms.

Experimental design

Outdoor mesocoms, or artificial ponds, were established in 80 l plastic tubs set up in a field at the University of Zurich, Switzerland. On 19 March 2010, 18 days before the experiment started, the tubs were filled with tap water, and stocked with 40 g dried leaf litter, 2 g rabbit chow and an inoculation of water and zooplankton from a natural freshwater pond. The tubs were covered with lids made of 43% shade cloth. The experimental design included 4 levels of salinity and a control, with 3 replicates. Based on previously reported salinity tolerance in *R. temporaria* (Viertel, 1999), we chose the following salt concentrations: 500, 1000, 2000 and 4000 mg/l, equaling chloride concentrations of 304 mg/l, 607 mg/l, 1214 mg/l, and 2428 mg/l, respectively. The salt concentration of the control (Zurich tap water without any addition of road salt or sodium chloride) was 100 mg/l, but we cannot be certain that the salt solute was solely sodium chloride. Salt concentrations were manipulated by adding the appropriate amount of deicing salt (sodium chloride (NaCl, 99.5%), sulfate (SO_4^{2-}, 0.5 %), ferrocyanide ($[Fe(CN)_6]^{4-}$, 20 mg/kg); Auftausalz 8300, Schweizer Rheinsalinen) to each experimental unit, and concentrations were carefully monitored using a salinometer (CDC401; Hach-Lange HQD series) measuring salt concentrations to the nearest 10 mg/l. The experiment also included a sixth treatment containing sodium chloride (NaCl; 71381, Fluka) at a concentration of 2000 mg/l, as we wanted to assess the effect of anti-caking agents in the deicing salt (ferrocyanide and sulfate). This concentration was chosen based on a reported mortality increase in *R. temporaria* tadpoles exposed to a sodium chloride concentration of 2300 mg/l (Viertel, 1999). The road salt treatment with a salinity of 2000 mg/l contained approximately 3.2 mg ferrocyanide per tub. Including the pure sodium chloride treatment, the experiment included a total of 18 mesocosms; treatment assignments were completely random.

Data collection

Several life history traits, i.e. growth, development, activity and survival, were chosen to assess the effect of salt on overall performance of tadpoles (Van Buskirk and McCollum, 2000; Altwegg and Reyer, 2003). To assess growth, body mass of tadpoles was recorded to the nearest mg on three dates during the experiment, on 26 April, 5 May and 14 May, when tadpoles were 29, 38 and 47 days old. Five tadpoles per tub were caught and weighed; this number was reduced if mortality was high. We concluded the experiment on 14 May, when tadpoles approached

metamorphosis, by counting and weighing all survivors, and staging them according to Gosner (1960). Behavior was observed on 28 April, when tadpoles were 31 days old. We visited each tub 6 times during the course of a day, and counted the number of visible animals that were active (swimming or feeding) and inactive (resting). Mortality rate was unknown at the time of observation, but the total number of tadpoles seen during behavior observations equaled in most instances the number of surviving tadpoles recovered at the end of the experiment.

Statistical analyses

To evaluate the effect of salinity on tadpole survival, we defined the response variable by the number of failures (number of dead tadpoles per tub) and the number of successes (number of surviving tadpoles per tub) and analysed the data using a generalized linear model with binomial errors. Activity data were translated into a single binary response variable containing 0s and 1s – 0 to represent inactive tadpoles, 1 to represent active tadpoles – and analysed using a generalized linear mixed model with binomial errors, with tub as a random effect. The tub effect was non-significant and was excluded from further analyses. A repeated measures analysis using a generalized linear mixed model with tub as a random effect was performed to model the effect of salinity on body mass; a linear model was used to evaluate the effect of salt on developmental stage. Mean values for each tub were used for both the analyses of body mass as well as developmental stage. Treatment (salinity) was included as a continuous factor in all models; in case of significant overall effects of treatment on response variables, the control treatment was specified as a reference group and all salt levels (included as categorical factor) were tested with the same model against the reference group to detect the lowest salt concentration that had an effect on the tadpole survival and performance. We were unable to compare the effects of road salt and pure sodium chloride on tadpole behavior, development and growth because few tadpoles survived at a salt concentration of 2000 mg/l.

Data analyses were performed in R 2.12 (www.r-project.org).

Results

Survival in the control treatment was (mean ± 1 SE) 97.2 % (± 2.78), decreasing significantly in the lowest salt concentration of 500 mg/l to a mean survival of 58.3 % (± 12.73; $z_{5,210}$= -3.011; P = 0.003) (Fig. 1). Overall, salinity strongly affected overall survival of larval *R. temporaria* ($z_{5,214}$= -6.272; $P \leq 0.001$). The impact on survival of pure sodium chloride at 2000 mg/l was not statistically different to that of road salt (sodium chloride plus anti-caking agents) treatment at the same salt concentration ($z_{5,210}$= -0.981; P = 0.327). Activity was influenced by road salt only at higher concentrations (Fig. 2): activity decreased from a mean of 37.5 % (± 0.28) in the control to an activity pattern where all tadpoles were either hiding or resting in the 2000 mg/l saline environment ($z_{5,488}$= -2.197; P = 0.028). When tadpoles were 47 days old, they were between Gosner stages 37 and 39, and there was no evidence that road salt affected the development ($t_{1,9}$= 0.762; P = 0.466). Growth was also not affected by road salt ($t_{9,22}$= 1.103; P = 0.299), and ranged between 0.03 (control, ± 0.002) and 0.04 (2000 mg/l road salt, ± 0.006) g body mass per day.

Discussion

Our study presents the effects of road salt on a European amphibian species in a semi-natural experimental setting, controlling for the effects of sodium chloride as well as anti-caking agents commonly contained in road salt. Larval *R. temporaria* growth and development was not affected by road salt, but tadpoles were adversely affected by all salinity levels tested: mortality increased at the lowest road salt concentration tested (500 mg/l road salt), and activity was affected at a road salt concentration of 2000 mg/l. Measured salinity levels in natural *R. temporaria* breeding ponds at the onset of its breeding season in 2011 have been found to range between 30 and 380 mg/l (Winkler, unpublished data), suggesting that these levels of salinity may be causing low to relatively high levels of larval mortality in the field, and may have no or little effects on larval behavior. However, our field measures of water salinity might underestimate salt levels in years with more snow fall and road salt application. Also, we might be underestimating the effects of our treatments on overall tadpole survival and other traits, as we only exposed tadpoles to increased salinities after hatching; embryonic stages may be even more sensitive to saline conditions than larval stages (Viertel, 1999). Overall, the evidence

suggests that managers should consider human salt input as a contaminant potentially putting amphibian species at risk, particularly in urban regions or areas close to roads.

When we compare the results from our experiment with those from other experimental studies evaluating the effect of sodium chloride on *R. temporaria* tadpoles, we see differences in outcome mainly regarding tadpole survival. Denoël and colleagues (2010) reared *R. temporaria* tadpoles in a laboratory experiment at sodium chloride concentrations ranging from 500 to 1500 mg/l, working with salinities overlapping with the range of salinities used in our experiment. Denoël et al. (2010) did not, however, detect increased tadpole mortality over the two-month study period. Similarly, Viertel (1999) detected increased mortalities of laboratory reared *R. temporaria* tadpoles only at relatively high sodium chloride concentrations: tadpole mortalities started to increase at sodium chloride concentrations of more than 2000 mg/l. Compared to a mortality of nearly 100% in our treatment of pure sodium chloride with approximately the same concentration, this is a surprising result. This could reflect the ability of *R. temporaria* to locally adapt to increased salt levels (Gomez-Mestre and Tejedo, 2003), as the population used for Viertel's (1999) experiment was sampled in a naturally salty environment with salt levels measured in breeding ponds as high as 900 mg/l; salt concentrations measured in the breeding pond where adult *R. temporaria* for our experiment were sampled were more than ten times lower. Also, the effect of venue should be considered as one of the reasons leading to different experimental outcomes, as both studies (Viertel, 1999; Denoël et al., 2010) were performed in the laboratory, while our study was conducted in a mesocosm setting.

We found that road salt had no effect on tadpole growth and development, and that tadpole activity was affected at a relatively high road salt concentration. Our results largely reflect the outcome of a study on the effect of road salt on tadpole behavior (Denoël et al., 2010): the authors showed that tadpole growth was not influenced by salt, but tadpole behavior was affected at a salt concentration of 1000 mg/l. However, the measure of activity (tadpole speed and distance) used in the behavioral study by Denoël and colleagues (2010) does not readily compare with our measures of activity, and might indicate that different behavioral traits may be affected differently by environmental stressors. Other confounding factors, such as the kind of salt used and the experimental setting, further obscure the comparability of our studies. Also, larval density is confounded with salinity levels in our experiment; we assume the presence of an effect of tadpole density on the outcome of our experiment, but are unable to quantify this

effect.

One of the main goals of our study was to compare the effects of road salt to those of pure sodium chloride. We expected that road salt would decrease tadpole performance even more than pure sodium chloride, because additional substances such as ferrocyanide are present in road salt. Instead, we observed no detectable difference between tadpoles exposed to commercial road salt and sodium chloride. This shows that additional substances in road salt do not strongly affect tadpoles at the relatively high concentration of 2000 mg/l. However, survival at this salt concentration was so poor that there was relatively little scope for detecting any effects of ferrocyanide. Future research should consider whether differences between road salt and sodium chloride emerge at lower salt concentrations.

Overall, our study shows that larval *R. temporaria* are relatively sensitive to road salt and are likely to suffer increased mortalities under salt concentrations measured in its natural habitat. We suggest that road salt should be considered as one of the factors putting amphibian species, *R. temporaria* in particular, at risk, and that wetlands close to roads should be monitored for salt contamination.

Acknowledgements

We thank Josh Van Buskirk, Corina Geiger, Owen Petchey, Benedikt Schmidt, Lorenzo Tanadini, Matteo Tanadini, Ursina Tobler and Valeria Rispoli for discussion and help, two anonymous reviewers for their constructive criticism, the Zürich Veterinary and Nature Conservation offices for permits, and the Swiss National Science Foundation for funding.

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Figure 1. Survival of *Rana temporaria* tadpoles under different concentrations of road salt (mean ± 1 SE, white points and bars). Grey transparent points represent mean tub values. The treatment depicted at a salt concentration of 100 mg/l refers to our control treatment, which did not include any added road salt, but showed a natural salt concentration of 100 mg/l. 'NaCl' refers to a pure sodium chloride treatment at a concentration of 2000 mg/l.

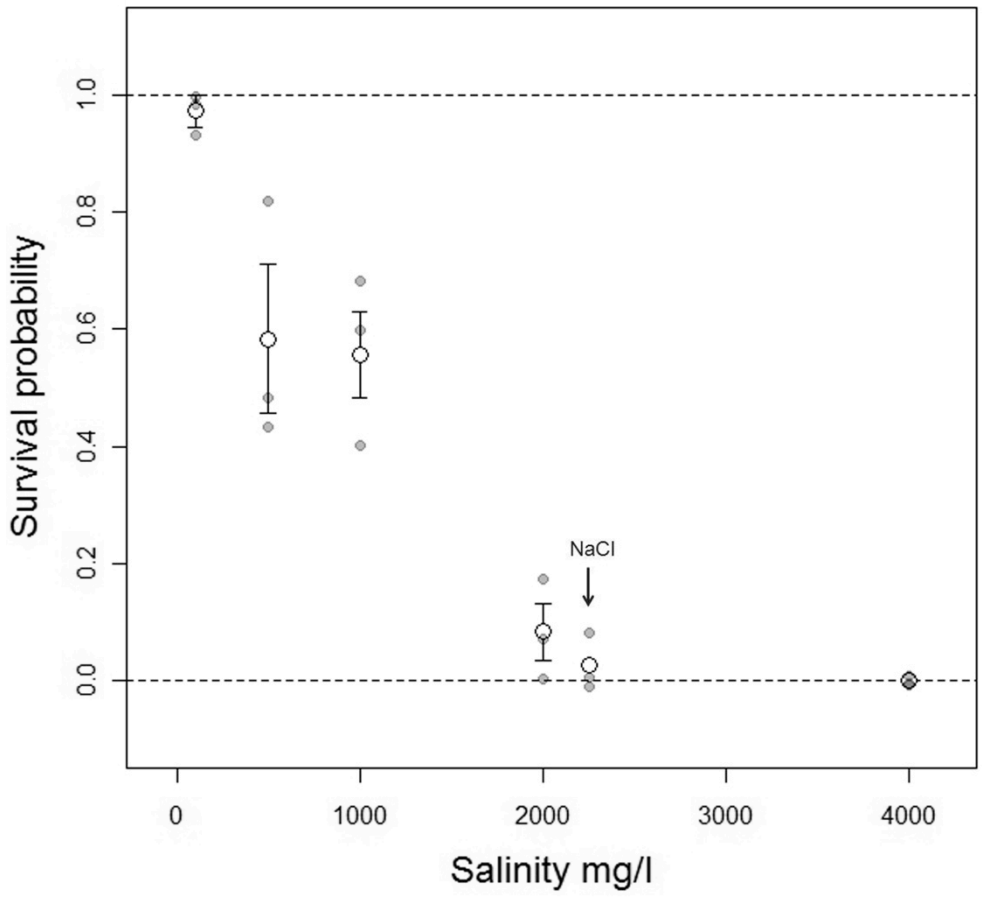
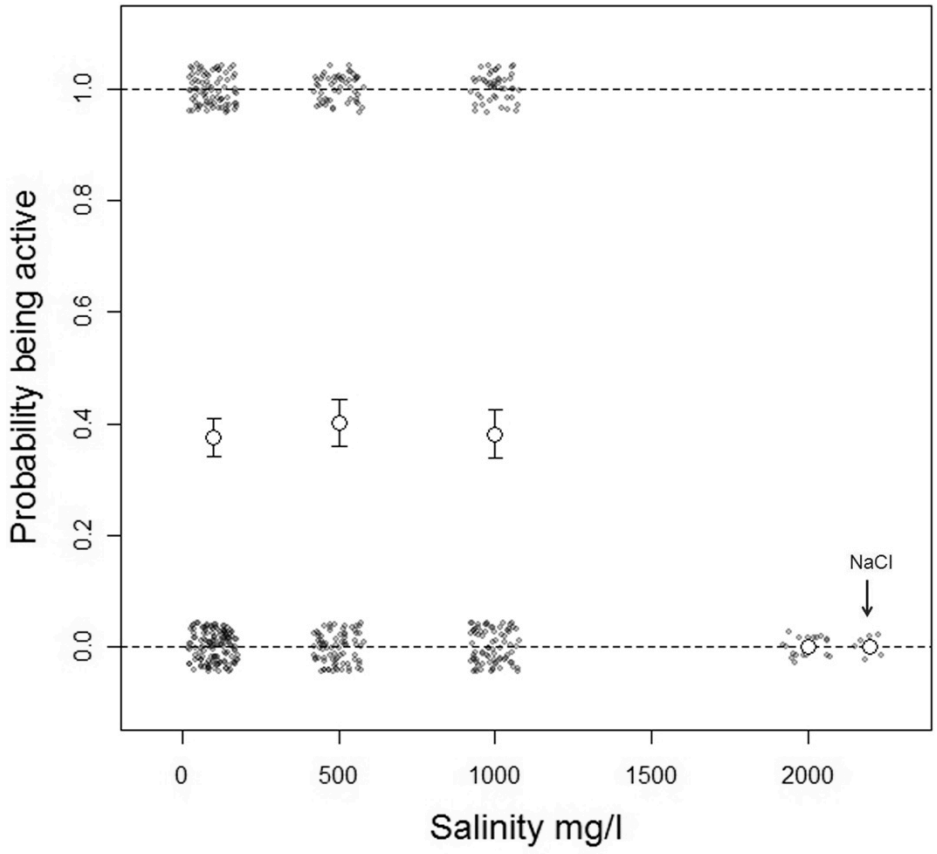


Figure 2. *Rana temporaria* tadpole activity under different concentrations of road salt (mean ± 1 SE, white points and bars), measured at age 31 days. Grey transparent points represent all data points collected in each treatment; each point clustered around value 1 of the y axis represents a tadpole that was active during an individual behavioral observation; each point clustered around value 0 of the y axis represents a tadpole that was inactive during an individual behavioral observation. The treatment depicted at a salt concentration of 100 mg/l refers to our control treatment, which did not include any added road salt, but showed a natural salt concentration of 100 mg/l. 'NaCl' refers to a pure sodium chloride treatment at a concentration of 2000 mg/l.



PREDICTING EVOLUTIONARY RESPONSE TO RAPID ENVIRONMENTAL CHANGE – GENETIC VARIATION OF A NATURAL POPULATION UNDER STRESS

Jasmin D. Winkler and Josh Van Buskirk

Abstract

Short-term evolutionary responses to changes in the environment can occur only if there is sufficient heritable variation in the population. While it is well known that genetic variation and adaptive potential are condition-dependent, it is still a major challenge to predict the implications of rapid environmental change for the evolutionary dynamics and future performance of natural populations. Several hypotheses predict how genetic variation should react to changes in the environment, but there is no consensus on the direction of change. Our study was designed to determine whether stressful environments lead to increased or decreased additive genetic variance (V_A), heritability (h^2), and Houle's evolvability (I_A), and whether the effect depends on the novelty or familiarity of stressful conditions. We reared larvae of the common frog *Rana temporaria* under semi-natural conditions in a large-scale outdoor experiment that included four different sources of stress: increased salinity and the presence of an agricultural pesticide (novel stressors), and apparent predation risk and high intraspecific competition (familiar stressors). Novel stressful conditions triggered an increase in additive genetic variance, heritability, and Houle's evolvability in some of the life history traits measured (survival and body weight at metamorphosis). This response would facilitate a rapid response to selection on those traits when novel conditions are encountered. However, stressful conditions that are commonly found in the natural habitat of *R. temporaria* did not lead to consistent changes in V_A, h^2 or I_A. Our data suggest that multiple mechanisms influence the expression of heritable variation, and that interpreting impacts of stress requires understanding of the species' ecology.

Introduction

The persistence of natural populations in a changing environment depends in part upon a species' adaptive potential, the ability to evolve in response to selection. Adaptive potential may be in turn influenced by changes in environmental conditions, as genetic variation expressed for the trait under selection can differ across environmental conditions (Hoffmann and Parsons 1991, Hoffmann and Merilä 1999, Charmantier and Garant 2005). While environmental fluctuations and heterogeneity can impose selection and are known to influence trait evolution (Hoffmann and Hercus 2000, Roff 2002), it is still a major challenge to predict the implications of a changing environment for the evolutionary dynamics and performance of wild populations (Millien et al 2006, Garant et al 2008). Our goal is to assess the effects of changing environmental conditions on genetic variation and adaptive potential in a natural amphibian population, and the implications of changing genetic variation for short-term evolutionary dynamics.

Trait heritabilities and variance components

A standard measure of the adaptive potential of continuous traits is narrow-sense heritability h^2 (Falconer and MacKay 1996), the proportion of the total phenotypic variance of a trait (V_P) that is due to additive effects of genes (V_A): $h^2 = V_A / V_P$. Heritability partly determines how rapidly the mean phenotype evolves in response to artificial or natural selection. Heritability is useful for the comparison of adaptive potentials across traits measured on different scales, because it is a ratio of variances of the same trait measured with the same units, and therefore dimensionless. Adaptive potential reflected by heritability refers to the expected change in a trait exposed to selection, expressed in standard deviation units of the trait prior to selection. For comparison across traits, V_A can also be scaled by the trait mean (m), a measure known as Houle's *I* (I_A), or evolvability: $I_A = V_A / m^2$ (Houle 1992). Here, the adaptive potential of a trait is realised as the expected percent change in a trait under a unit selection differential. It is evident that h^2 and I_A are uncorrelated and have led to different conclusions (Houle 1992, Hansen et al 2011), and it has been argued that h^2 is especially poorly suited as a measure of the adaptive potential in traits closely related to fitness (Houle 1992). Thus, we are considering both h^2 and I_A to assess the effects of changing environmental conditions on adaptive potential.

It is well known that levels of V_A and h^2 can depend on environmental conditions, but outcomes from laboratory and field studies disagree about whether exposure to unfavourable or stressful conditions leads to an increase or decrease in variance components and heritabilities. Evidence from laboratory experiments suggests that genetic variation and heritabilities increase under stress (Hoffmann and Parsons 1991, see Hoffmann and Merilä 1999 for a review). But this trend does not seem to apply to more natural situations. A few studies of wild populations have shown that unfavourable conditions trigger a decrease in heritability, mostly due to an increase in the environmental fraction (V_E) of the total phenotypic variance (Réale et al 1999, Merilä and Sheldon 2001, Uller et al 2002, Garant et al 2004). However, a broader review shows that these are exceptions; overall, there is little evidence that h^2 consistently decreases under stressful conditions in field studies (Blanckenhorn 2002, Charmantier and Garant 2005). Evolvability is not commonly reported in quantitative genetic studies, and the current literature does not allow for a comprehensive comparison of I_A estimates obtained in different venues. A number of laboratory studies report increased evolvabilities under stressful conditions (e.g. Imasheva et al 1999, Willi et al 2010, Willi et al 2011), but evidence from field studies is too sparse to report a comprehensive pattern. Overall, predicting stress-dependent changes in evolvability is a challenge because environment-sensitivity of both V_A and the phenotypic trait means must be considered. Both are known to be responsive to both stressful conditions and venue (Hoffmann and Parsons 1991, Winkler and Van Buskirk 2012).

The source of the discrepancy between estimated effects of stress on genetic variation in laboratory and field venues is unknown. Several characteristics of lab and field conditions have been suggested to be important. For example, experimental approaches have mostly been limited to the study of laboratory reared inbred lines that have been exposed to stressors that are rarely found in nature, and are generally more extreme than those applied or naturally present in studies investigating wild populations. Also, the laboratory environment itself may be novel, potentially leading to the expression of sets of genes that had previously not been exposed to natural selection, inflating V_A and heritability estimates (Holloway et al 1990, Hoffmann and Parsons 1991). Environmental quality, however, can also be problematic in studies of wild populations, as it is often difficult to characterise natural conditions. In both field and lab studies it can be a challenge to judge how benign or stressful any given environment

really is, making it even more difficult to qualify the outcome of both venues for studying the influence of changing conditions on adaptive potential.

Another challenge when studying the environment-dependency of I_A and h^2 is that the estimates obtained in any particular environment are specific to that environment, constraining attempts to generalise about predicted trait evolution across other environments or taxa (Steppan et al 2002). What is needed is an experimental setup where environmental conditions can be controlled and simulated, but which at the same time resembles natural conditions as close as possible. Our study aims to identify a common pattern of change in the adaptive potential of a large set of traits when exposed to multiple stressful conditions, focusing on a wild population of the common frog *Rana temporaria* in a semi-natural environment. In particular, we tested whether the change in genetic architecture triggered by stressful environmental conditions depends on whether those conditions are common (herein referred to as familiar stressors) or exceptional (referred to as novel stressors) in nature. As outlined above, the lack of concordance between results obtained from laboratory studies and studies on wild populations might be explainable by differences in novelty of the environments under study. Based on predictions derived from the collection of earlier studies reviewed by Hoffmann and Merilä (1999), and Charmantier and Garant (2005), we predict that V_A will increase when organisms are presented with a novel stressor, leading to increased heritability, as well as an increase in I_A, given that trait means remain relatively stable. In contrast, we predict that familiar stressors will lead to reduced trait heritabilities due to an increase in V_E; I_A is insensitive to changes in V_E, and will only differ from benign conditions if trait means change under stress.

Material and Methods

The common frog *R. temporaria* is particularly well suited for this study, as this amphibian species allows for the application of quantitative genetic breeding designs and controlled mating. Also, the aquatic environment of its larval stage can easily be manipulated, and a number of life history and morphological traits are known to have a heritable genetic basis in certain environments (Sommer and Pearman 2003, Laugen et al 2005). We reared larvae under semi-natural conditions in a large-scale outdoor experiment that included four different sources

of stress: increased salinity, presence of an agricultural pesticide, apparent predation risk and high intraspecific competition. These four treatments are known to be stressful at some levels for *R. temporaria* larvae (Viertel 1999, Teplitsky et al 2005, Stamper et al 2008, Steiner & Van Buskirk 2008, Winkler and Forte 2011), and are known to influence the expression of an array of fitness-related life history traits. Increased salinity levels and the presence of a pesticide represent novel stressors for *R. temporaria*, because amphibian populations in our study area are rarely and only very recently in contact with those agents. In contrast, predation risk and intraspecific competition are part of the natural environment of *R. temporaria*, and here considered as familiar stressors.

Artificial crosses

The crossing design was an incomplete North Carolina II (Lynch & Walsh 1998, p. 599), repeated in 4 blocks of parents (Figure 1). There were 60 crosses in all, sired by 20 males each crossed with 3 different females, and 20 females each crossed with 3 different males. We collected 28 *Rana temporaria* males and 24 females in March 2010 from a large breeding population in Canton Schaffhausen, Switzerland (47°36'N, 8°40'E). Artificial crosses were performed in the laboratory the following day, largely following the protocol from Räsänen and colleagues (2003; Hangartner 2010). Male *Rana temporaria* were injected with synthetic luteinizing hormone-releasing hormone to stimulate sperm production (fish LHRH H-7525, Bachem AG), and sperm were harvested about two hours later by flushing the cloaca with 10 % Amphibian Ringer solution (Rugh 1962). We stripped eggs from females, divided egg masses among three containers for each female, and covered eggs with sperm solution from a single male. Fertilized eggs were flooded with Ringer solution and kept outdoors until hatching. In the end, 11 of the 60 crosses failed, so that we chose 45 crosses sired by 17 males and 16 females for the experiment. Six of the sires had offspring with only 2 females, and 3 of the dams had offspring with only 2 males.

Experimental Design

Tadpoles from each of the 45 full-sib families were subjected to five treatments: a control representing benign conditions, and four stressful treatments. There was one replicate of each treatment-family combination, for a total of 225 experimental units. The experiment occurred in

mesocosms (plastic tubs: 80-L, 0.28 m^2) set up in an open field at the University of Zurich. We filled mesocosms with tap water on 1-3 March 2010, 34-36 days before the start of the experiment. Mesocosms were covered with 43% shade cloth lids and stocked with 40 g of dried leaf litter, 2 g of rabbit food, and additional water and zooplankton from a nearby pond.

The salt treatment was established by adding 1722 g of Sodium chloride (71381, Fluka) to each mesocosm; final salinity was adjusted to 2.8 ppt (± 0.04) using a salinometer (CDC401; Hach-Lange HQD series). Eight days after the experiment started, we reduced salinity to 2.0 ppt (± 0.03) due to relatively high early mortality. On 29 April, salinity was measured a second time and only minor adjustments had to be made (departure from original salinity was on average 0.05 ± 0.02 ppt). Each mesocosm in the pesticide treatment received 960 µg fenpropimorph (36772, Sigma-Aldrich) by adding 2.1 ml of a 0.46 mg / ml pesticide / acetone solution one day after the tadpoles were introduced, resulting in a starting concentration of 12 µg / l. Fenpropimorph is commonly used in Switzerland to control fungal pests in cereals and sugar beet, is typically applied once or twice per year to agricultural fields (0.75 kg / ha), and has been detected in natural streams in Switzerland at low concentrations (0.21 µg / l; www.awel.zh.ch). Considering an estimated half-live of approximately 64 days in water, and 54 days in sediment (Tomlin 1997), pesticide concentrations in our experiments probably declined to around 6 µg / l when tadpoles started to metamorphose. We simulated high predation risk in one treatment by confining a single *Anax imperator* dragonfly larva inside a cage within each mesocosm, and feeding it 300 mg of *Rana temporaria* tadpoles that were not part of our study three times a week throughout the experiment. Cages were not present in the other four treatments. The competition treatment contained 45 tadpoles per mesocosm, three times higher than the 15 individuals present in all other treatments. The control treatment did not receive any further adjustments or manipulations. Tadpoles were added to the experiment on 5 April, when they were four days old (stage 23; Gosner 1960). We removed tadpoles from the mesocosms when they reached stage 42 (forelimb emergence), and held them individually in the laboratory until they reached complete tail resorption at stage 45.

Measuring traits

Life history. – Body mass was recorded to the nearest mg on 22 April, when tadpoles were 21 days old. On this occasion, five individuals per mesocosm were caught, weighed, and

immediately returned. We recorded survival, body mass, and age at Gosner stage 45 for all individuals.

Behavior. – We observed behavior on 11 May, when tadpoles were 40 days old. We visited each experimental unit six times and counted the number of visible animals that were active (swimming or feeding) and inactive (resting). Tadpoles not visible to the observer were scored as hiding and assumed to be inactive.

Morphology. – The five tadpoles that were caught and weighed on 22 April were also photographed from above in a water-filled petri dish. Photographs were used to measure the following body dimensions: distance between the tip of the nose and the distal end of tail fin (hereafter referred to as total length), and maximum body width. Length measurements from each photograph were recorded using a digital image analysis program.

Quantitative genetic analyses

Phenotypic variances were decomposed into additive genetic (V_A), maternal (V_M; including maternal inheritance and maternal environmental effects) and residual components (V_R). V_R represents V_E, but also dominance (V_D) and epistatic (V_I) effects, which cannot be distinguished from V_E with our breeding design. However, V_D and V_I are likely much smaller than V_E, and we will assume that V_R represents mostly V_E. We estimated variance components separately for each trait and treatment from a mixed model using Bayesian inference, including sire and dam as random effects and no fixed effects. 95% credible intervals were computed for h^2, I_A, and each variance component, and estimates from the control and stressful treatments were considered to be significantly different from each other when their 95% credible intervals were not overlapping. To test whether single trait additive genetic variances and maternal effects were significantly different from zero, mixed models where only the sire or dam effect was specified were computed using restricted maximum likelihood (REML), and compared with a full model (including both sire and dam as random effects) using a likelihood ratio test. Trait heritabilities and evolvabilities were considered to be significantly different from zero when traits showed significant levels of V_A. Due to high mortality, only survival was analysed in the salt treatment.

To be able to directly compare variance components across traits measured on different scales (Table 2), phenotypic data were centered and scaled to unit variance. Unscaled data were used

to estimate I_A, and h^2 estimates calculated from unscaled data were identical to h^2 estimates obtained from scaled data. Analyses were performed in R 2.12 (www.r-project.org), employing the packages lme4 and MCMCglmm (Bates 2005, Hadfield 2010).

Results

Mean values for all traits and all environments are listed in Table 1.

Heritability and additive genetic variance were low but significant for most traits in most environments (Table 2). Heritabilities (given as h^2 with lower and upper range of credible intervals) were highest for tadpole body dimensions in the competition treatment (total length: 0.39, 0.23-0.67; body width: 0.32, 0.15-0.56), and lowest for behavioural traits and survival in almost all treatments. Evolvability (Houle's I / I_A) was highest for behavioural traits in multiple conditions, and lowest for age at metamorphosis, body weight at metamorphosis and measurements of body dimensions (total length and body width); other traits showed a mix of relatively high and low I_A's among the different treatments (Table 2). Heritability under stressful conditions differed from the benign control treatment in several cases. Two out of eight traits showed significant changes under stress, recognized by non-overlapping credible intervals in control and stress treatments (body weight at metamorphosis in the pesticide treatment, survival under salt stress). This is a fairly stringent criterion for identifying a change due to stress, so we also considered cases in which heritability changed by a factor of three or more; these are indicated with arrows in Table 2. Stressful conditions induced both increases and decreases in h^2, but whenever the change was significant, it was an increase in h^2 under a novel stressor. Body weight at metamorphosis and activity (both swimming and feeding) were the traits that were most sensitive to environmental conditions, showing more than three-fold increases or decreases in h^2 under stress. Significant changes in heritability were due to changes in V_A as well as V_R (mostly representing V_E). Maternal variation was often different from zero, but was never significantly affected by environmental conditions. Stressful conditions triggered strong but non-significant changes in V_M in four instances, including the same two changes under novel stressors as recorded for V_A (body weight at metamorphosis in the pesticide treatment, survival under salt stress; Table 2).

Evolvabilities were similarly affected by stressful conditions as heritabilities. I_A significantly increased under novel stressors (body weight at metamorphosis in the pesticide treatment, survival under salt stress), just as it was the case for h^2. Also, body weight at metamorphosis and activity (feeding) were the two traits that were most sensitive to stress, showing strong increases as well as decreases in I_A. Evolvability and heritability were uncorrelated (Spearman's rank correlation coefficient: *rho* = 0.103; *S* = 5370; *P* = 0.569), but changes in I_A and h^2 in response to stressful conditions showed a significant correlation (Spearman's rank correlation coefficient: *rho* = 0.64; *S* = 936; *P* < 0.001).

Discussion

We predicted that novel conditions would lead to an increase in heritability due to increasing V_A, while stressful conditions that are not novel would lead to a decrease in trait heritability due to increasing V_E. Indeed, whenever the change in heritability was significant in our experiment, this change - an increase in h^2 associated with an increase in V_A - was induced by novel conditions. These changes involved mass at metamorphosis in the pesticide treatment and survival in the salt treatment. Heritability did not significantly decrease under novel conditions for any trait. These results indicate that the potential to respond to environmental stressors differs between familiar and novel stressful conditions, but that this difference is pronounced only in a few life history traits. The affected life history traits will be able to respond faster to selection under novel stressors than under familiar stressors.

It has been argued that novel stressful conditions lead to the expression of higher levels of additive genetic variation in traits related to fitness compared to environments that are commonly encountered by organisms, because in novel or rarely encountered environments selection has not yet removed alleles associated with lower fitness (Holloway et al 1990). Our results support this hypothesis, which yields specific predictions about changes of V_A and h^2 under stress. However, as the results of our study are only phenomenological, there could be multiple reasons behind the observed changes, and other mechanisms involved.

Survival and mass at metamorphosis, two traits closely related to fitness in amphibians (Altwegg and Reyer 2003), both showed increasing genetic variation under novel stress. However, age at

metamorphosis, a trait that is also related to fitness in amphibians (Altwegg and Reyer 2003), did not show the same pattern of change under novel conditions. Apparently, the mechanism suggested by Holloway and colleagues (1990) does not apply to all fitness-related traits in our experiment.

Stressful conditions that were not novel, i.e. predation risk and competition for resources, did not lead to a change in heritability in any consistent direction. Overall, it seems that stressful conditions that are common in nature may trigger appreciable changes in V_A, V_E and h^2 but in unpredictable directions. However, body weight at metamorphosis represents an exception to this pattern: V_A increased in the presence of predators as well as competitors, elevating h^2 estimates more than three- and two-fold, respectively. This contradicts our prediction that h^2 would decrease under the influence of a familiar stressor, but might be explainable by interactions between environmental and genetic effects expressed under resource limitation. The relationship between enzyme activity and flux - the reaction velocity through metabolic pathways - is non-linear: the lower the enzyme activity, the greater the impact of enzyme activity on flux. Under resource limitation, where enzyme activity is relatively low, phenotypic differences between genotypes in flux can become apparent (Hartl et al 1985, Hoffmann and Parsons 1991, Ward 1994), and could possibly explain the observed increase in h^2 for body weight at metamorphosis in the competition and predation treatments. Under competition resources are severely limited, and under predation tadpoles reduce their feeding activities to a minimum to avoid being detected by a potentially lethal predator, which results in reduced rates of resource intake and growth in tadpoles (Van Buskirk 2000). The resource-limited conditions caused by competition and predation risk may therefore enhance genotypic differences among tadpoles in gaining body weight, possibly involving digestive physiology. The same hypothesis might serve as an explanation for the observed, more than two-fold increase in h^2 of total length and body width in the competition treatment.

Predation risk also triggered a tripling of levels of V_A in feeding behavior, leading to an almost three-fold change in heritability compared to the control treatment. Resource limitation cannot explain this result because the same pattern did not appear in the competition treatment. Gavrilets and Scheiner (1993) proposed another hypothesis explaining stress-dependent changes in trait heritabilities: h^2 might be influenced not only by genetic variation of the trait itself, but also by genetic variation for plasticity in the trait. Under this scenario, heritability can change

dramatically with changing environmental conditions, given that genes controlling plasticity and phenotypic trait means are strongly associated, and heritability for plasticity is high. The heritability for plasticity in feeding behavior is unknown in our study organism, but activity is highly plastic in *R. temporaria* (Van Buskirk 2001), and Gavrilets and Scheiner's (1993) mechanism might be applicable in this case. To further confirm this hypothesis, environmental dependency of the genetic architecture of other traits that are known to plastically respond to the presence of predators, such as tail depth, should also be studied in amphibians.

Heritabilities and evolvabilities were uncorrelated in our study. This agrees with previous comparisons of the two measures across many studies (Houle 1992, Hansen et al 2011). In spite of this, the changes in I_A induced by stress were roughly the same as changes in h^2, under both novel and familiar stressors. It is difficult so far to evaluate the generality of this observation, because evolvability is reported relatively infrequently in quantitative genetic studies. Houle (1992) and others have argued that studies of the environment-dependency of adaptive potential should report I_A estimates along with h^2. Nevertheless, this result does suggest that genetic mechanisms underlying change in evolvabilities and h^2 may be similar.

We aimed to interpret the outcome of our study in the context of several hypotheses that have been used to explain environment-dependent changes in V_A and h^2 (e.g. Hoffman and Merilä 1999). However, not all observations made here were suitable for testing these hypotheses. For example, under the resource limitation model, we expected all fitness traits to show an increase in h^2 under stress; in other words, survival, age at metamorphosis, and body weight at metamorphosis should have shown the same pattern of change. This was not observed. One problem with our expectation is that the adaptive potential of any single trait may not be independent of other traits under selection, as pleiotropy or linkage disequilibrium can lead to genetic correlations among traits (Lande and Arnold 1983, Roff 1997), which are themselves sensitive to environmental conditions (Rose 1984, Holloway et al 1991). Patterns of change in variance components in response to stressful environmental conditions are likely interconnected among traits, obscuring the patterns of change emerging in studies such as ours, where genetic trait components and their environment-dependent changes are considered independently from each other. Little is known about the stability and condition-dependency of the G-matrix - the matrix of genetic variances and covariances - in nature (Steppan et al 2002, Jones et al 2003).

This should be addressed in further studies that aim to understand the condition-dependency of h^2 and I_A.

Our results suggest that several mechanisms influence the adaptive potential under novel and familiar stressful conditions in the common frog. We propose that the ecology of the species must be carefully considered when studying how stressful environmental conditions affect the adaptive potential. This supports conclusions made in similar studies investigating the consistency of genetic variance components among venues (e.g. Blanckenhorn 2002).

Acknowledgements

We would like to thank Corina Geiger, Morana Mihaljević, Oscar Ramos, Lorenzo Tanadini, Ursina Tobler, and Iris Wurmitzer for help with the experiment. Thanks to Andrew Beckerman, Wolf Blanckenhorn, Corina Geiger, Owen Petchey, Eric Postma, Katja Räsänen, Uli Reyer, Lorenzo Tanadini, Matteo Tanadini, and Ursina Tobler for discussion and statistical help, to the Zürich Veterinary and Nature Conservation offices for permits, and to the Australian Research Council and Swiss National Science Foundation for funding.

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Table 1. Mean trait values (± SD) and number of individuals measured or number of mesocosms contributing to the estimate (n) in a benign (control) and four stressful treatments.

	Body weight at metamorphosis (mg)		Body weight at age 21 days (mg)		Age at metamorphosis (days)		Total length at age 21 days (mm)		Body width at age 21 days (mm)	
	x (± SD)	*n*	*x* (± SD)	*n*	*x* (± SD)	*n*	*x* (± SD)	*n*	*x* (± SD)	*n*
Control	344.8 (50.0)	461	190.9 (52.0)	225	64.6 (2.7)	461	26.3 (2.5)	219	6.2 (0.5)	221
Predation	363.4 (53.0)	435	104.9 (34.5)	225	73.1 (3.1)	435	20.7 (2.1)	220	5.2 (0.5)	223
Competition	139.6 (36.3)	1660	186.1 (48.6)	225	72.7 (8.2)	1160	25.9 (1.7)	220	6.1 (0.5)	219
Pesticide	342.1 (50.9)	452	174.4 (40.2)	225	65.0 (3.3)	452	25.5 (1.9)	219	6.0 (0.5)	218
Salinity	451.1 (98.5)	177	163.7 (52.7)	116	65.9 (3.6)	177	25.1 (2.8)	113	5.8 (0.6)	109

	Survival until Gosner stage 42 (%)		Activity at age 40 days (number of tadpoles feeding)		Activity at age 40 days (number of tadpoles swimming)		Activity at age 40 days (total %)	
	x (± SD)	*n*	*x* (± SD)	*n*	*x* (± SD)	*n*	*x* (± SD)	*n*
Control	93.6 (10.7)	45	2.9 (2.1)	45	1.6 (1.4)	45	45.6 (9.4)	45
Predation	90.7 (12.2)	45	0.8 (1.4)	45	1.1 (1.5)	45	28.7 (7.8)	45
Competition	91.7 (7.7)	45	10.3 (4.7)	45	4.5 (2.7)	45	56.4 (7.2)	45
Pesticide	93.7 (7.7)	45	3.0 (2.2)	45	1.7 (1.6)	45	45.4 (7.5)	45
Salinity	35.0 (38.4)	45	0.7 (1.4)	29	0.6 (1.0)	29	22.5 (14.3)	29

Table 2. Houle's *I*, heritabilities and variance components for all phenotypic traits measured. Bold numbers are I_A and h^2 estimates, and variance components that are significantly different from zero. Arrows indicate whether I_A and h^2 estimates or variance components showed a more than three-fold increase (arrow pointing upwards), or decrease (arrow pointing downwards) compared to the control treatment. Underlined numbers indicate a significant change compared the control treatment. Variance components are estimates from standardized data.

	I_A				
	Control	**Predation**	**Competition**	**Pesticide**	**Salinity**
Body weight at metamorphosis	0.0004	**0.0019** ↑	**0.0032** ↑	**0.0050** ↑	-
Body weight at age 21 days	**0.0134**	**0.0218**	**0.0172**	**0.0047**	-
Age at metamorphosis	**0.0004**	**0.0002**	**0.0009**	**0.0004**	-
Total length	**0.0012**	**0.0013**	**0.0017**	**0.0007**	-
Body width	**0.0014**	**0.0014**	**0.0017**	**0.0004** ↓	-
Activity (swimming)	**0.0553**	0.0230	**0.0225**	0.0056 ↓	-
Activity (feeding)	**0.0271**	**0.5634** ↑	0.0017 ↓	0.0094	-
Survival	0.0016	0.0007	0.0021	0.0004 ↓	**0.6560** ↑

Table 2 continued.

	h^2				
	Control	**Predation**	**Competition**	**Pesticide**	**Salinity**
Body weight at metamorphosis	0.02	**0.11** ↑	**0.05**	**0.21** ↑	-
Body weight at age 21 days	**0.18**	**0.25**	**0.31**	**0.14**	-
Age at metamorphosis	**0.21**	**0.09**	**0.08**	**0.16**	-
Total length	**0.15**	**0.14**	**0.39**	**0.09**	-
Body width	**0.14**	**0.12**	**0.32**	**0.09**	-
Activity (swimming)	**0.06**	0.01 ↓	**0.04**	0.01 ↓	-
Activity (feeding)	**0.05**	**0.14**	0.01 ↓	0.01 ↓	-
Survival	0.02	0.01	0.03	0.01	**0.25** ↑

Table 2 continued.

	V_A				
	Control	**Predation**	**Competition**	**Pesticide**	**Salinity**
Body weight at metamorphosis	0.01	**0.12** ↑	**0.05** ↑	**0.20** ↑	-
Body weight at age 21 days	**0.17**	**0.25**	**0.19**	**0.14**	-
Age at metamorphosis	**0.24**	**0.10**	**0.08** ↓	**0.15**	-
Total length	**0.15**	**0.12**	**0.46** ↑	**0.08**	-
Body width	**0.19**	**0.10**	**0.37**	**0.08**	-
Activity (swimming)	**0.06**	0.01 ↓	**0.04**	0.01 ↓	-
Activity (feeding)	**0.05**	**0.13**	0.01 ↓	0.01 ↓	-
Survival	0.02	0.02	0.02	0.01	**0.28** ↑

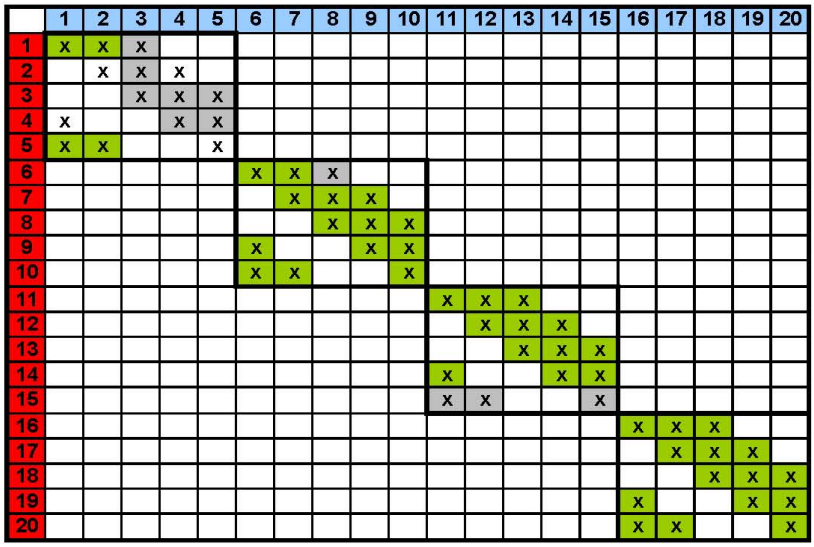
Table 2 continued.

	V_R				
	Control	**Predation**	**Competition**	**Pesticide**	**Salinity**
Body weight at metamorphosis	**0.91**	**0.83**	**0.93**	**0.67** ↓	-
Body weight at age 21 days	**0.39**	**0.44**	**0.42**	**0.32**	-
Age at metamorphosis	**0.58**	**0.83**	**0.80**	**0.69**	-
Total length	**0.55**	**0.49**	**0.47**	**0.36**	-
Body width	**0.46**	**0.57**	**0.46**	**0.35**	-
Activity (swimming)	**0.90**	**0.94**	**0.98**	**0.96**	-
Activity (feeding)	**0.90**	**0.76**	**0.93**	**0.89**	-
Survival	**0.94**	**0.96**	**0.94**	**0.98**	**0.63**

Table 2 continued.

	V_M				
	Control	**Predation**	**Competition**	**Pesticide**	**Salinity**
Body weight at metamorphosis	**0.04**	**0.05**	**0.06**	**0.24 ↑**	-
Body weight at age 21 days	**0.31**	**0.20**	**0.27**	**0.29**	-
Age at metamorphosis	**0.21**	**0.02 ↓**	**0.10**	**0.10**	-
Total length	**0.12**	**0.21**	**0.10**	**0.23**	-
Body width	**0.24**	**0.18**	**0.21**	**0.40**	-
Activity (swimming)	**0.01**	0.01	0.01	0.02	-
Activity (feeding)	**0.02**	**0.04**	0.02	**0.11 ↑**	-
Survival	**0.02**	0.01	**0.02**	0.01	**0.15 ↑**

Figure 1. Mating design in which each male *R. temporaria* (blue numbers) was crossed with three females (red numbers), and each female was crossed with three males. Males and females were arranged into four blocks of parents. Each cross between a male and a female is marked with a cross; green background indicates crosses used for this study, grey background indicates crosses that failed to develop, and white background indicates crosses that successfully developed, but were not used for this study. The design is based on a North Carolina II mating design.



ACTIVITY AND PERFORMANCE PATTERNS BETWEEN COMPETITION AND PREDATION ENVIRONMENTS IN A SPECIES OCCUPYING A HABITAT GRADIENT

Jasmin D. Winkler and Josh Van Buskirk

Abstract

Community structure and the distribution of coexisting species among habitats can be explained by the interaction of predation and competition. This interaction is commonly believed to be shaped by a trade-off between a species' ability to avoid predators and its qualities as a competitor, and arises when specific traits that confer high fitness under competition for resources are costly in the presence of predators. A predation-competition trade-off ultimately restricts the distribution of a species to habitat conditions that match its competitive ability or its facility at avoiding predators, a phenomenon that is known to exist in a number of systems. We tested whether the mechanisms that enforce a trade-off among species operates within species, expressed as a genetic correlation between traits reflecting competitive ability and the ability to escape predation. Using a quantitative genetic breeding design, we crossed adults of the common frog *Rana temporaria*, and reared 45 sibships of larvae in an outdoor mesocosm experiment where apparent predation risk and competitor density was manipulated. Larval behavioural activity levels, developmental time, growth rates, and an overall performance measure, which was a function of both developmental time and growth rate, were scored for each family, and analysed using quantitative genetic methods. Cross-environmental genetic correlations were indistinguishable from zero for three of the four traits measured, despite significant levels of additive genetic variances for the majority of traits measured in the two environments. However, body weight at metamorphosis was positively correlated between the high competition environment and the environment where tadpoles were exposed to apparent predation risk. Overall, the lack of negative cross-environmental genetic correlations between traits reflecting competitive abilities and predator-avoidance abilities implicates that *R. temporaria* is not a polymorphic generalist, where genotypes are specialised to perform well only in one of the environments. At the same time, our results do not exclude the presence of a competition-predation trade-off among genotypes of this species.

Introduction

An interaction between predation and competition is often invoked to explain community structure and the distribution of species among habitats (Connell 1961, Werner and Anholt 1993, Wellborn et al 1996, Viola et al 2010). This interaction is believed to be shaped by a trade-off between the ability of a species to compete for resources and to avoid predators, restricting the distribution of a species to habitat conditions that match its competitive ability or its facility at avoiding predators. Empirical data on a number of systems demonstrate the existence of such a trade-off. For example, anuran species typically replace each other along a habitat gradient ranging from permanent ponds, where predator densities are high and competition for resources is low, to temporary puddles that contain fewer or no predators but higher densities of competitors (Woodward 1982, Wellborn 1996, Van Buskirk 2003). Species inhabiting temporary waters are described as being more vulnerable to predators, but superior competitors, compared to permanent pond species (Woodward 1982, Lawler 1989, Kruuk and Gilchrist 1997). Other taxa exhibiting evidence of a trade-off between the ability to compete for resources and avoid predators include odonate larvae, freshwater amphipods, plants, and microbes (McPeek 1990, McPeek 1996, Wellborn 2002, Franks et al 2008, Jessup and Bohannan 2008).

A predation-competition trade-off arises when specific traits that confer high fitness under competition for resources are costly in the presence of predators (e.g. Futuyma and Moreno 1988, Jaenike 1990), due to antagonistic pleiotropy or linkage disequilibrium. One trait that has been proposed to mediate this trade-off is general behavioural activity level. Species that are very active are often effective at gathering resources and growing rapidly, but they can be less tolerant of predators because of enhanced encounter rates (Werner and Anholt 1993, Stoks et al 2003). In principle, the same mechanisms that enforce a trade-off among species could operate within species. That is, just as we find an interspecific predation-competition trade-off – mediated by activity or any other trait – a similar mechanism could occur within generalist species that are exposed at times to high densities of either competitors or predators. This intraspecific predation-competition trade-off could occur at two levels: distinct populations could locally adapt to habitats with high densities of either predators or competitors, or genotypes within populations could show different levels of specialization in their competitive

ability or ability to escape predation. This paper focuses on the second level, which is essentially a polymorphic generalist type described by Levene (1953) and others (Moran 1992). We address the following question: is the predation-competition trade-off present within populations?

An evolutionary trade-off between competition and predation is manifest in genetic variation in the ability of individuals to avoid predators and compete with conspecifics, and a negative genetic correlation between the two abilities (Futuyma & Moreno 1988). In other words, an unavoidable trade-off occurs when genotypes that perform well with predators do poorly under competition. In this case, the genetic correlation is between the same character (fitness) measured in two environments and treated as two different traits (Falconer 1952). The same approach can be applied to non-fitness traits, assuming the traits have known performance consequences in situations involving predator-avoidance and competition. For example, traits related to foraging effort may be positively associated with competitive ability, and general behavioural activity may be negatively associated with survival under predation risk. A positive cross-environmental genetic correlation between foraging effort under competition and activity under predation could then be interpreted as evidence for a predation-competition trade-off.

Our approach to detecting the existence of a predation-competition trade-off within populations was twofold. First, we tested for the activity-based trade-off between competitive ability and resistance to predation by comparing the levels of behavioural activity in the presence of competitors to those in the presence of predators of a large number of genotypes. The study animals were larvae of the common frog, *Rana temporaria*, an anuran species that occurs along a continuum of predator and competitor environments (Van Buskirk 2003). This species responds to both competitors or predators by changing behaviour adaptively: competitors induce higher foraging activity, and predators induce lower overall activity levels (Van Buskirk 2002, Teplitsky and Laurila 2007). In this context, we inferred that a genotype had high competitive ability if it showed a relatively high level of activity in the presence of competitors. This follows from the observation that individual tadpoles tend to increase foraging effort when exposed to food shortage or competitors (Anholt et al 2000, Relyea 2004, Teplitsky and Laurila 2007, Raffel et al 2010), and species of anurans with high competitive ability are relatively active during the larvae stage (Smith and Van Buskirk 1995, Lindgren and Laurila 2010). Likewise, we equated a relatively low activity under predation risk with high resistance to predators. This, too,

is because intra- and inter-specific comparisons indicate that greatly reduced activity is associated with higher survival when hunting predators are present (Anholt and Werner 1998, but see Van Buskirk et al 1997). A predation-competition trade-off occurs if genotypes with an activity response conferring high competitive ability also have a response to predators conferring poor ability to resist predation, i.e. genotypes that show relatively high levels of activity under high densities of competitors also exhibit relatively high levels of activity under predation risk. A trade-off is then realised as a positive genetic correlation between behavioural responses induced by predators and competitors.

Our second test for the predation-competition trade-off was based on the performance of tadpoles when challenged with competitors or predation risk, estimated as a function of both age at metamorphosis and metamorphic body weight. The rationale was similar to that described for a trade-off mediated by activity. A genotype was inferred to have high competitive ability if competitors had a weak negative effect on performance, i.e. if performance was relatively high under competition, but relatively low under predation. In the same way, high resistance to predation was inferred if performance declined relatively little when predators were present, i.e. if performance was relatively high under predation risk. Under this scenario, a trade-off appears as a negative cross-environmental genetic correlation of performance traits, i.e. genotypes that show relatively high performance under competition show relatively low performance under predation.

Material and Methods

To evaluate whether an activity- or performance-mediated trade-off exists between predator avoidance and the ability to tolerate high densities of competitors, we reared sibships of *R. temporaria* larvae in an outdoor experiment where apparent predation risk and competitor density was manipulated, and used quantitative genetic methods to estimate levels of additive genetic variation and genetic correlations between treatments in behavioural activity and tadpole performance. The common frog is well suited to study the genetic underpinnings of the interaction between predation and competition within populations because *R. temporaria* tadpoles are known to respond to both predators and competitors by changing behavioural

activity levels (Van Buskirk 2002, Teplitsky and Laurila 2007), which in turn influence tadpole traits that are related to fitness, namely larval development time and growth rates (Van Buskirk 2000, Winkler and Van Buskirk 2012). Also, this species is amenable to artificial breeding designs, allowing us to confidently estimate genetic variation and genetic correlations using quantitative genetic methods.

Artificial crosses

In March 2010 we collected 28 *Rana temporaria* males and 24 females from a large breeding population in Canton Schaffhausen, Switzerland (47°36'N, 8°40'E), and performed artificial crosses largely following Räsänen (2003; Hangartner 2010). Eggs were stripped from each female, divided among three containers, and each container was covered with sperm solution from a single male that was previously injected with synthetic luteinizing hormone-releasing hormone to stimulate sperm production. Fertilized eggs were flooded with 10% Amphibian Ringer solution (Rugh 1962) and kept outdoors until hatching. The crossing design was an incomplete North Carolina II (Lynch & Walsh 1998, p. 599), repeated in four blocks of five parents each (Fig. 1). This involved 20 males each crossed with three different females, and 20 females each crossed with three different males, producing 60 crosses in total. This design allowed us to estimate additive genetic variances and covariances in traits related to competitive ability and predator avoidance. Eleven of the crosses failed, so the experiment included 45 successful crosses sired by 17 males and 16 females. Six sires had offspring with only 2 females, and 3 of the dams had offspring with only 2 males.

Experimental Design

Tadpoles from each of the 45 full-sib families were subjected to three experimental treatments: control, predation, and competition. There was one replicate of each treatment-family combination, for a total of 135 experimental units. The experiment occurred in mesocosms (plastic tubs: 80-L, 0.28 m^2) set up in an open field at the University of Zurich. Each mesocosm was filled with tap water and covered with 43% shade cloth lids 34-36 days before the start of the experiment. Mesocosms were stocked with 40 g of dried leaf litter, 2 g of rabbit food, and additional water and zooplankton from a nearby pond.

Predation risk was simulated by adding a single *Anax imperator* dragonfly larva to each mesocosm assigned to the predation treatment. Dragonfly larvae were kept in plastic cages, unable to prey upon the experimental tadpoles, and received controlled feedings of 300 mg of *R. temporaria* tadpoles that were not part of our study three times a week throughout the experiment. Cages were not present in the other two treatments. The mesocosms assigned to the competition treatment contained 45 tadpoles each (161/m^2), compared to 15 individuals in all other treatments (54/m^2). The control treatment did not receive any further adjustments or manipulations. All tadpoles entered the experiment on 5 April, at the age of four days (stage 23; Gosner 1960). Experimental tadpoles were removed from the mesocosms when they reached Gosner stage 42 (forelimb emergence), and kept individually in the laboratory until they reached complete tail resorption at Gosner stage 45.

Measuring traits

We observed behavior on 11 May, when tadpoles were 40 days old. We visited each experimental unit six times and counted the number of visible animals that were active (swimming or feeding) and inactive (resting). Tadpoles not visible to the observer were scored as hiding and assumed to be inactive. In addition to tadpole behavior, we recorded two traits of metamorphosing froglets that are together indicative of individual performance during the preceding larval stage and the subsequent terrestrial stage. Body mass to the nearest mg at Gosner stage 45 (herein referred to as body weight at metamorphosis) is associated with adult survival, size, and age at first reproduction in anurans (Smith 1987, Berven 1990, Altwegg and Reyer 2003). Age at metamorphosis, the number of days each tadpole required to reach Gosner stage 45, is also correlated with survival and age at first reproduction (Smith 1987, Altwegg and Reyer 2003). Tadpole performance was therefore a function of both age and mass at metamorphosis. We integrated these two components using a relationship between survival to one year of age and size/age at metamorphosis estimated by Altwegg and Reyer (2003) for *Rana esculenta* and *R. lessonae*: logit[survival probability] = -0.69 + 0.87 * [body weight at metamorphosis] – 0.021 * [age at metamorphosis]. We measured age and body weight at metamorphosis in the same units as Altwegg and Reyer (age in days and body weight in SD units), and used model parameters estimated by Altwegg and Reyer. This analysis assumes that selection on metamorphic life-history traits acts similarly in *R. temporaria* and the species

studied by Altwegg and Reyer (2003). The resulting estimated survival to age 1 is what we hereafter refer to as "performance." Larval survival was not included in this measure because it was not comparable between treatments. Survival in the competition treatment was directly influenced by competitors, whereas the presence of a caged dragonfly larva in the predation treatment did not affect tadpole survival. In principle, one could estimate the effects of predation on tadpole survival with experiments in which predators are unrestrained.

Competitive ability and resistance to predators were measured by activity, performance-related traits and performance in the competition and predation treatments. These values were scored or calculated for each sibship and subjected to quantitative genetic analyses to reveal the genetic architecture of the predation-competition trade-off. We used a Wilcoxon rank sum test with continuity correction to find out whether there was a significant difference between trait values, and performance between treatments and the control.

Quantitative genetic analyses

Phenotypic variances and covariances were decomposed into additive genetic (V_A), maternal ($V_{M,}$ including maternal inheritance and maternal environmental effects), and residual components (V_R). The residual mostly represents environmental variance (V_E). We estimated variance components separately for each combination of treatment (competition, predation) and trait (activity, age and body weight at metamorphosis, performance) from a mixed model using restricted maximum likelihood (REML), including sire and dam as random effects, and no fixed effects. To test whether additive genetic variances and maternal effects of traits and changes in traits were significantly different from zero, mixed models where only the sire or dam effect was specified were computed using REML, and compared with a full model (including both sire and dam as random effects) using a likelihood ratio test.

Bivariate mixed models using Bayesian inference were computed to estimate the genetic covariance between activity, performance and performance-related traits under the exposure to competition and predation. Cross-environmental genetic correlations were computed as $r_A = COV_{Apc} / (V_{Ap} / V_{Ac})^{0.5}$, where subscripts p and c represent the predation and competition environments. Genetic correlations range between -1 and +1; the sign reflects whether traits are positively or negatively correlated across environments, and the magnitude reveals how strong

the correlation is. Genetic correlations were considered to be significant when 95% credible intervals did not overlap zero (Wilson et al 2010). Genetic variances and covariances were similar when estimated by REML.

Phenotypic data were centred and scaled to unit variance to be able to directly compare variance components across traits measured on different scales (see Table 1). Analyses were performed in R 2.12 (www.r-project.org), employing the packages lme4 and MCMCglmm (Bates 2005, Hadfield 2010).

Results

Mean values for all traits and performance are listed in Table 1.

Competition and predation treatments led to changes in activity and both performance-related life-history traits (Table 1). Compared with those in the control treatment, tadpoles increased levels of activity when they were exposed to high densities of competitors (W = 1746.5, P < 0.001), and were less active in the predation treatment (W = 325, P < 0.001). Tadpoles needed 13% more time to reach metamorphosis when they developed under predation risk (W = 1988, P < 0.001) or at high densities of competitors (W = 2022, P < 0.001). The competition treatment led to a 60% decrease in body weight at metamorphosis (W = 0, P < 0.001), whereas metamorphs were about 5% heavier when they developed under predation risk (W = 1321.5, P = 0.013). Individual performance, computed as a function of both age and body weight at metamorphosis, did not differ between the predation and the control treatments (W = 863.5, P = 0.23), because the opposing changes in mass and development rate roughly balanced each other. In contrast, under competition tadpoles suffered greatly decreased levels of performance compared to control tadpoles (W = 0, P < 0.001).

There was significant additive genetic variance and maternal variance in performance and the two performance components in most treatments, but not for activity (Table 1). However, there was little evidence for genetic correlations − negative or positive − between effects of predators and competitors (Table 1). Only one trait – body weight at metamorphosis – showed a barely

significant positive cross-environmental genetic correlation (0.64), indicating that genotypes that grew heavy under predation risk also metamorphosed with a relatively high body weight under high densities of competitors. Other genetic correlations were close to zero.

Discussion

The restricted distributions of species along important ecological gradients (predation risk, conspecific density, habitat permanence) are often thought to be enforced by a trade-off between competitive ability and success at avoiding predators. Indeed, there are many examples in which species that perform well under resource limitation experience high mortality rates under predation (e.g. Woodward 1982, McPeek 1996, Franks et al 2008). We investigated whether this trade-off also exists within species, expressed as a genetic correlation between traits reflecting competitive ability and the ability to escape predation measured across two environments with manipulated densities of conspecifics and non-lethal predators. There were significant amounts of genetic variation for most traits associated with the ability to tolerate a high competition environment as well as the presence of a predator, indicating that genotypes differed in how they were affected by the different conditions (Table 1). However, we did not find a positive cross-environmental genetic correlation of activity, or a negative cross-environmental genetic correlation of performance measures. This suggests that *R. temporaria* is not a polymorphic generalist, where different genotypes are specialized in predator-avoidance or competition. Treatment specific developmental rates and levels of performance were genetically uncorrelated between the two environments, but yielded significant amounts of V_A, indicating that traits can independently respond to different selection regimes along a habitat gradient. This outcome further suggests that most of the genes controlling those traits under high competition differ from those that determine trait expression under predation risk (Falconer 1981), or that gene regulation is independent among environments. Body weight at metamorphosis showed a different pattern: this trait was positively correlated between the competition and predation environment; genotypes that metamorphosed with relatively high body mass under predation risk showed a tendency to also metamorphose relatively heavy in a high competition environment. The positive cross-environmental genetic correlation implies that

selection on metamorphic body weight in one of the two environments facilitates a positively correlated response in metamorphic body weight in the other environment (Roff 1997). Also, genes determining metamorphic body weight in an environment where intraspecific competition is high are probably mostly the same genes that regulate metamorphic body weight under predation risk (Falconer 1981). Levels of V_A for activity were not different from zero in all treatments measured, and so were cross-environmental genetic correlations. Considering that activity levels are known to affect tadpole survival when hunting predators are present, and can influence the competitive ability of tadpoles (Werner and Anholt 1993, Stoks et al 2003), it is possible that past selection drove the genes controlling activity levels to fixation in this population, leading to the observed low levels of V_A. In any case, based on non-significant amounts of V_A in activity in both competition and predation environments, this trait is not able to directly respond to selection along a predation-competition gradient.

It is important to search for trade-offs among genotypes within populations, as the presence or absence of such trade-offs illuminate the evolutionary dynamics of the population under investigation. Population structure of generalist species is classically believed to be shaped by fitness trade-offs between ecological conditions that can lead to processes of genetic divergence across different environmental regimes, forming disparate populations that evolve to specialize on situations where either predation risk or competition for resources are present (e.g. Futuyma and Moreno 1988, Castillo-Chavez et al 1988, Jaenike 1990). These populations might in turn give rise to distinct species that partition the predator-competition gradient. Based on the absence of specialised genotypes for either one of the environments, the population investigated here does not fall within the classical view of how generalists are shaped by trade-offs experienced through environmental heterogeneity. There is no indication that this population is currently undergoing processes of genetic divergence. However, Fry (1996) pointed out that negative genetic correlations across environments might not be necessary to enforce specialisation within habitats. In his model, cross-environmental genetic correlations that are less than one mean that the rank-order of genotypes on the performance scale is environment-specific, i.e. the highest-ranking genotype under predation risk is not the highest-ranking genotype under high competition. According to Fry (1996) this can sometimes be enough to promote specialisation. Effectively, Fry's scenario can result in locally specialised populations that eventually give rise to separate species across a predator-competition gradient, just as a

predation-competition trade-off within populations is predicted to promote. In other words, cross-environmental genetic correlations that are either negative (e.g. Futuyma and Moreno 1988, Jaenike 1990), or any value that is different from one (Fry 1996) can eventually lead to genetic divergence and specialisation. All cross-environmental genetic correlations estimated in this study fall within this range of values, and the trade-off hypothesis cannot be rejected based on the absence of negative cross-environmental genetic correlations, or positive cross-environmental genetic correlations whenever contrasting selection regimes are present in different conditions.

Based on current theory, the outcome of our study also allows for a different interpretation: the absence of a classical predation-competition trade-off has been implicated in two certain types of generalists. According to Levins (1968) the 'jack-of-all-trades' generalist consists of individuals that show intermediate performance across all conditions encountered. Due to the absence of genotypes that are specialised in any environment, we expect to find that traits are genetically uncorrelated across conditions, just as are the majority of the traits measured in *Rana temporaria*. The other type of generalist, the so-called habitat-generalist (McPeek 1996), has also been associated with the absence of a genetically based predation-competition trade-off. McPeek (1996) argued that the classical perception of generalists being shaped by fitness trade-offs between ecological conditions might have exceptions, for example when environmental heterogeneity affects specialists and generalists differently, preventing a fitness trade-off in the generalist species that is able to find its niche in different communities. The habitat generalist occupies a broad range of disparate habitats and is insensitive to environmental heterogeneity, and is predicted to coexist with closely related specialists or other types of generalists (McPeek 1996). The distribution of our study species, *R. temporaria*, overlaps with the habitat of species that are more specialised to certain environmental conditions, for example the common toad *Bufo bufo* (Van Buskirk 2003). Considering the habitat distribution of the common frog, its coexistence with specialist species, and the fact that we did not find a trade-off between disparate ecological conditions, it seems possible that this species is a habitat generalist *sensu* McPeek (1996). Parallel studies with coexisting specialists are needed to illuminate how the common frog is affected by heterogeneous environments, and how this compares to the tolerance of coexisting specialist species to different environmental conditions. In summary, the results of our study could be interpreted in several ways; depending on the model used, our

data can either point toward *Rana temporaria* harbouring the potential to genetically diverge across a habitat gradient, or being a habitat-, or 'jack-of-all-trades' generalist that is well adapted to heterogeneous conditions.

An alternative interpretation of our results is that a fitness trade-off actually does exist between performance in environments with high predation risk and many competitors, but that it is not detectable under our experimental conditions. It is conceivable that body weight at metamorphosis underlies contrasting selection regimes in the two conditions measured, which would translate the positive cross-environmental genetic correlation into a trade-off mediated by a performance related life-history trait. Although larval growth rates – and ultimately metamorphic body size – are believed to be under positive selection in an environment with high competition as well as under predation risk (e.g. Travis 1980), selection on developmental time might be increased in temporary ponds where competition for resources is often high, changing selection regimes along a predation-competition gradient. Our findings also cannot exclude trade-offs in *Rana temporaria* that include predation from non-dragonfly species (e.g. newts), or parasites and pathogens, which are known to play a great role in other systems (e.g. Gwynn et al 2005).

Acknowledgements

We would like to thank Corina Geiger, Morana Mihaljević, Oscar Ramos, Lorenzo Tanadini, Ursina Tobler, and Iris Wurmitzer for help with the experiment. Thanks to Andrew Beckerman, Wolf Blanckenhorn, Corina Geiger, Eric Postma, Katja Räsänen, Uli Reyer, Lorenzo Tanadini, Matteo Tanadini and Ursina Tobler for discussion and statistical help, to the Zürich Veterinary and Nature Conservation offices for permits, and to the Australian Research Council and Swiss National Science Foundation for funding.

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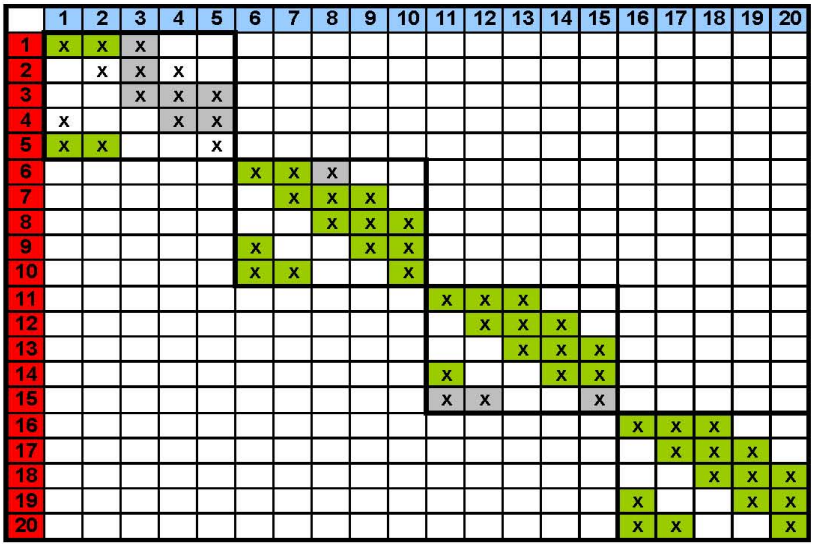
Table 1. Summary of mean trait values (top) and estimates of genetic variance components (middle) in the three treatments, and genetic correlations between the predation and competition treatments (bottom). Performance is the estimated survival to age 1, based on age and mass and metamorphosis (see Methods). Sample size (*n*) is the number of individuals measured or used for calculations. Va is the additive genetic variance among sires (V_A), Vm is the maternal variance (V_M), and V_R is the residual variance. Boldface highlights variance components and genetic correlations that are significantly different from zero.

	Activity (proportion of time)			**Body weight at metamorphosis (mg)**			**Age at metamorphosis (days)**			**Performance**		
	x (± SD)		*n*	*x* (± SD)		*n*	*x* (± SD)		*n*	*x* (± SD)		*n*
Control	0.46 (0.22)		45	344.8 (50.0)		461	64.6 (2.7)		641	0.27 (0.08)		641
Predation	0.29 (0.30)		45	363.4 (53.0)		435	73.1 (3.1)		435	0.27 (0.08)		435
Competition	0.56 (0.19)		45	139.6 (36.3)		1660	72.74 (8.2)		1160	0.06 (0.02)		1160
	V_A	**V_M**	**V_R**	**V_A**	**V_M**	**V_R**	**V_A**	**V_M**	**V_R**	**V_A**	**V_M**	**V_R**
Control	<0.001	0.01	0.99	0.01	**0.04**	**0.9**	**0.24**	**0.21**	**0.6**	0.03	0.04	**0.9**
Predation	0.03	<0.001	0.99	**0.12**	**0.05**	**0.8**	**0.10**	**0.02**	**0.8**	**0.1**	**0.06**	**0.8**
Competition	<0.001	<0.001	0.99	**0.05**	**0.06**	**0.9**	**0.08**	**0.10**	**0.8**	**0.13**	**0.09**	**0.8**
Cross-environmental genetic correlations (95% credible intervals)	0.03	(-0.50 – 0.46)		**0.64**	**(0.01 – 0.99)**		-0.07	(-0.65 – 0.54)		-0.02	(-0.44 – 0.49)	

Figure 1. Mating design in which each male *R. temporaria* (blue numbers) was crossed with three females (red numbers), and each female was crossed with three males. Males and females were arranged into four blocks of parents. Each cross between a male and a female is marked with a cross; green background indicates crosses used for this study, grey background indicates crosses that failed to develop, and white background indicates crosses that successfully developed, but were not used for this study. The design is based on a North Carolina II mating design.



SUMMARY

Summary

The interaction between an organism and its environment has substantial ecological and evolutionary implications. In heterogeneous environments, physical and biotic factors change over time and space, exposing animals and plants to stressful conditions. Those conditions, understood as external factors that impair fitness, can affect biological systems at various levels, from individuals, through populations and species to ecosystems.

Understanding the direct effects of stressful conditions on an individual level yields important ramifications for nature conservation and management. If stress is detrimental to individual fitness, this can in turn influence population dynamics and impact the evolutionary processes that influence the fate and long-term persistence of populations and species. This thesis aims to contribute to our understanding of the effects of stressful environmental conditions on individual and population levels using an amphibian species, the common frog *Rana temporaria*, as a study system.

Chapter 1

Experiments are commonly used to answer ecological and evolutionary questions in biology, but the choice of the experimental venue can influence the outcome of the experiment, implicating the drawn conclusions for answering the study question. When assessing the effects of stressful conditions on natural organisms, it is of particular importance that the insights gained from any experiment can be translated to natural conditions.

In my first chapter I set out to compare the outcome of the same ecological experiment performed in the three most commonly used venues in experimental ecology and evolutionary biology: the laboratory, mesocosms (outdoor artificial ponds combining characteristics of the lab with that of nature), and the field. As the choice of venue is often associated with trade-offs, I also aimed to weigh realism against precision and replication across all venues in order to evaluate which specific needs of the experimenter are met by what kind of experimental setting.

To compare experimental outcome and evaluate the trade-off associated with different venues,

I measured the effects of venue on larval traits of *Rana temporaria* tadpoles. In all three venues, the experiment exposed tadpoles to conditions with and without a caged *Anax imperator* dragonfly larva. Predation risk is a stress that is commonly encountered by *R. temporaria* tadpoles in their natural environment, and is well suited to study the effect of venue on the reaction to stressful conditions. To evaluate how realistic the outcome of any venue is, the experimental tadpoles were compared with wild tadpoles that originated from the same population.

I was able to show that the experimental venue influenced nearly every trait I measured, although some of the traits were more sensitive to venue than others. While laboratory environment triggered highly distinctive morphology, tadpoles in mesocosms were most similar to those in field enclosures and the wild. This suggests that mesocosms are well suited to study the effects of stressful conditions in an ecological and evolutionary context.

Chapter 2

My second chapter was dedicated to study the influence of a stressor that has only very recently entered the environment of the common frog: road salt, a chemical compound that is targeted for human use and unintentionally contaminates freshwater, lakes and drinking water. To date, road salt has received relatively little attention for its detrimental effects on the environment, but its direct effects on tadpole life history and behaviour have important implications for species conservation and nature management.

A mesocosm experiment with five different salt concentrations revealed that tadpoles showed decreased survival and activity at increased salinities. Contrasting my expectations, mortality rates under road salt did not differ from those observed in a sodium chloride control lacking the toxic substance ferrocyanide found in the deicer. Road salt caused increased mortality even at the smallest concentration tested, and behavioral activity levels of the surviving tadpoles were only impacted at a higher salt concentration.

Chapter 3

Environmental stressors not only have direct effects on individuals and populations, but can also be seen as an evolutionary force. To date, the evolutionary impacts of environmental stress are not entirely clear, and it is a major challenge to predict the implications of stressful conditions for the evolutionary dynamics and future performance of populations. Short-term evolutionary responses to changes in the environment can occur only if there is sufficient heritable variation in the population. However, we know relatively little about how stressful conditions affect the amount of genetic variation present in a population and how this relates to the evolutionary potential of that population. While it is well known that genetic variation and adaptive potential are condition-dependent, there is no consensus on the direction of change, resulting in part from discrepancies in the outcome of laboratory and field studies. Some characteristics of both laboratory and field studies have been suggested to be important when explaining the differences in outcome among the two venues, including differences in novelty of the applied stressors.

In my third chapter, I assessed how genetic variation and evolutionary potential is affected by stressful environmental conditions under the aspect of the novelty of the applied stressors. My study was designed to determine whether stressful environments lead to increased or decreased additive genetic variance (V_A), heritability (h^2), and Houle's evolvability (I_A), and whether the effect depends on the novelty or familiarity of stressful conditions. To answer this question, I reared common frog larvae that originated from artificial crosses in a large-scale mesocosm experiment that included four different sources of stress: increased salinity and the presence of an agricultural pesticide (novel stressors), and apparent predation risk and high intraspecific competition (familiar stressors). Using quantitative genetic methods, I was able to show that novel stressful conditions triggered an increase in additive genetic variance, heritability, and Houle's evolvability in some of the life history traits measured. In a broader context, the outcome of this experiment suggests that those traits that have been observed to change their adaptive potential under stress can respond more rapidly to selection when novel conditions are encountered. However, stressful conditions that are commonly found in the natural habitat of *R. temporaria* did not lead to consistent changes in V_A, h^2 or I_A. The outcome of this experiment suggests that multiple mechanisms influence the expression of heritable variation, and that interpreting impacts of stress requires understanding of the species' ecology.

Chapter 4

The idea of fitness trade-offs is central to most theories for the evolution of ecological specialization, and is believed to explain processes determining community structure, species distributions and specialization to certain types of habitat. A trade-off can arise, if a species exhibits traits that confer high fitness under one of the commonly encountered environmental conditions, but that are costly under another environment.

A trade-off between a species' ability to avoid predators and its qualities as a competitor is often assumed to explain species communities and distributions along predation-competition gradients, limiting the distribution of a species to habitat conditions that match its competitive abilities or its ability to avoid predators. Empirical data on a number of systems demonstrate the existence of such a trade-off. For example, anuran species typically replace each other along a habitat gradient ranging from permanent ponds, where predator densities are high and competition for resources is low, to temporary puddles that contain fewer or no predators but higher densities of competitors.

In my fourth chapter I set out to assess whether the predation-competition trade-off is also present on an intraspecific level, among genotypes of a population that inhabits a region where individuals encounter both high densities of predators as well as competitors. In theory, a population could yield genotypes that are specialized in avoiding predators, as well as genotypes that are particularly well suited to compete for resources. Just as it is being found among closely related species, genotypes that are good competitors are expected to be bad at avoiding predation, and a trade-off will appear as a genetic correlation of the traits that mediate the predation-competition trade-off. Relative performance in both environments, and levels of behavioral activity are traits that can mediate such a trade-off in the common frog.

To observe whether genotypes present in a population of the common frog differ in their abilities to avoid predators and compete for resources, I crossed adult common frogs and reared their offspring in an outdoor mesocosm experiment. The experiment was set up in a way that the environment of each tadpole sibship was manipulated for apparent predation risk and competitor density. Larval behavioural activity levels, developmental time, growth rates, and an

overall performance measure were scored for each sibship, and analysed using quantitative genetic methods.

I was able to establish that traits were not genetically correlated across environmental conditions in three of the four traits measured, despite significant levels of additive genetic variances for the majority of traits. However, body weight at metamorphosis was positively correlated between the high competition environment and the environment where tadpoles were exposed to apparent predation risk. The results of my study indicate that genotypes of the common frog are not specialised to be either good competitors, or good at avoiding predators. However, the results of my study do not exclude the possibility that a competition-predation trade-off exists in the common frog.

ZUSAMMENFASSUNG

Zusammenfassung

Die Interaktion zwischen einem Organismus und seiner Umwelt ist von tiefgreifender ökologischer und evolutionsbiologischer Bedeutung. In heterogenen Umgebungen ändern sich physikalische und biotische Faktoren über Zeit und Raum, was für Tiere und Pflanzen Stress bedeuten kann. Stressoren - externe Faktoren, die organismische Fitness beeinträchtigen - können biologische Systeme auf verschiedenen Ebenen beeinflussen: von Individuen, über Populationen und Arten, bis hin zu Ökosystemen.

Untersucht man die direkten Auswirkungen ungünstiger Bedingungen auf der Ebene von Individuen, so kann das wichtige Erkenntnisse für den Natur- und Artenschutz liefern. Beeinträchtigt Stress organismische Fitness der Individuen einer Population, so kann dies wiederum Einfluss auf die Populationsdynamik nehmen und evolutionäre Prozesse beeinflussen, die die langfristige Fortdauer von Populationen und Arten bestimmen. Meine Arbeit zielt darauf ab die Auswirkungen stressiger Umweltbedingungen zu beleuchten – sowohl auf der Ebene von Individuen, als auch auf der Ebene von Populationen. Der Grasfrosch *Rana temporaria* ist dabei mein Studienobjekt.

Kapitel 1

Um ökologische und evolutionäre Fragen zu beantworten wählt man häufig experimentelle Ansätze. Allerdings kann die Wahl der experimentellen Versuchsumgebung das Ergebnis des Experiments beeinträchtigen, was wiederum die mittels des Experiments gezogenen Schlussfolgerungen zur Beantwortung der Studienfrage beeinflusst. Möchte man die Auswirkungen von ungünstigen Bedingungen auf natürliche Organismen untersuchen, so ist es von besonders grosser Bedeutung, dass die aus einem Experiment gewonnene Erkenntnis auf natürliche Bedingungen übertragen werden kann.

Das Ziel meines ersten Kapitels war es die drei in der Ökologie gebräuchlichsten Versuchsumgebungen miteinander zu vergleichen. Dazu habe ich das gleiche ökologische Experiment im Labor, in Mesokosmen (im Freiland angelegte, künstliche Teiche, die Laboreigenschaften mit denen aus dem Feld kombinieren), und dem Feld durchgeführt.

Schliesslich habe ich die Effekte quantifiziert, die die jeweilige Versuchsumgebung auf das Versuchsergebnis hatte. Da die Wahl der Versuchsumgebung häufig mit Kompromissen verbunden ist, habe ich ausserdem Realismus, Präzision und Replikation aller Versuchsumgebungen miteinander verglichen, um beurteilen zu können welche spezifischen Bedürfnisse des Experimentators durch welche Art von experimenteller Umgebung erfüllt sind.

Um die Versuchsergebnisse miteinander zu vergleichen, mass ich die Auswirkung der Versuchsumgebung auf verschiedene larvale Merkmale des Grasfrosches. Im Rahmen des Experiments wurden Kaulquappen einem Prädationsrisiko ausgesetzt, das durch die Präsenz oder Absenz einer eingesperrten, und somit nicht tödlichen *Anax imperator* Libellenlarve manipuliert wurde. Prädationsrisiko ist ein Stress, der häufig in der natürlichen Umgebung von Grasfrosch Kaulquappen angetroffen wird, und ist gut dazu geeignet, die Interaktion zwischen Versuchsumgebung und Stress zu untersuchen. Um beurteilen zu können wie realistisch das Versuchsergebnis einer jeden Versuchsumgebung ist, wurden die experimentellen Kaulquappen mit wilden Kaulquappen aus derselben Population verglichen.

Ich konnte zeigen, dass die experimentelle Versuchsumgebung fast jedes der gemessenen Merkmale beeinflusst, obwohl einige der Merkmale empfindlicher gegenüber der Versuchsumgebung waren als andere. Während die Laborumgebung eine sehr distinkte Kaulquappenmorphologie hervorrief, ähnelten die Kaulquappen aus den Mesokosmen denjenigen aus dem Feldversuch und der freien Wildbahn. Meine Resultate deutet darauf hin, dass Mesokosmen gut dazu geeignet sind die Auswirkungen von ungünstigen Bedingungen in einem ökologischen und evolutionären Kontext zu untersuchen.

Kapitel 2

Mein zweites Kapitel ist dem Einfluss eines Stressors gewidmet, der erst in jüngster Zeit in die natürliche Umgebung des Grasfrosches getreten ist: Streusalz, eine chemische Substanz, die für den menschlichen Gebrauch bestimmt ist und unabsichtlich Süßwasser, Seen und Trinkwasser verunreinigt. Bis heute haben die schädlichen Auswirkungen von Streusalz relativ wenig Aufmerksamkeit erhalten, obwohl seine direkten Auswirkungen auf die Entwicklung,

Lebensdauer und das Verhalten von Kaulquappen und anderen Organismen bedeutsam für den Arten- und Naturschutz sind.

Ein Mesokosmosexperiment mit fünf verschiedenen Salzkonzentrationen hat gezeigt, dass ein erhöhter Salzgehalt das Überleben von Kaulquappen verringert, und ihre Aktivität beeinflusst. Entgegen meinen Erwartungen hat das Experiment ausserdem gezeigt, dass sich die Sterblichkeitsrate unter dem Einfluss von Streusalz nicht von derjenigen unterscheidet, die ich unter dem Einfluss von Natriumchlorid beobachten konnte, welches nicht die im Streusalz enthaltene toxische Substanz Ferrocyanid beinhaltet. Streusalz führte bei der kleinsten getesteten Konzentration zu erhöhter Kaulquappenmortalität; höhere Salzkonzentrationen führten zu Verhaltensstörungen bei den überlebenden Kaulquappen.

Kapitel 3

Umweltstressoren haben nicht nur direkte Auswirkungen auf Individuen und Populationen, sondern können auch als evolutionäre Kraft gesehen werden. Bis heute sind die evolutionären Auswirkungen von Umweltstress nicht ganz klar, und es ist eine große Herausforderung die Auswirkungen von ungünstigen Bedingungen für die evolutionäre Dynamik und zukünftige Entwicklung von Populationen vorherzusagen. Kurzfristige evolutionäre Antworten auf Umgebungsveränderungen können nur dann auftreten, wenn eine ausreichende erbliche Variation in der Population vorhanden ist. Allerdings wissen wir relativ wenig darüber, wie stressige Bedingungen den Anteil an genetischer Variation in einer Population beeinflusst, und sich somit auf das Potenzial zur evolutionären Anpassung auswirken. Während es allgemein bekannt ist, dass sich genetische Variation und das Potenzial zur evolutionären Anpassung in Abhängigkeit von der Umwelt verändern kann, gibt es bisher keinen Konsens darüber in welche Richtung diese Veränderungen zielen. Ein Teil dieser Ungewissheit geht aus dem Phänomen hervor, dass Labor- und Feldstudien oftmals unterschiedliche Versuchsergebnisse hervorbringen. Einige Eigenschaften dieser beiden Versuchsumgebungen könnten Aufschluss über die Zusammenhänge zwischen Umwelt und evolutionärem Potenzial geben, darunter Unterschiede in der Neuartigkeit der verwendeten Stressoren in den Labor- und Feldstudien.

In meinem dritten Kapitel habe ich den Einfluss von Umweltstress auf die genetische Variation und das evolutionäre Potenzial einer Grasfroschpopulation untersucht, speziell unter dem Blickwinkel der Neuartigkeit von verschiedenen untersuchten Stressoren. Meine Studie wurde so entworfen, dass ich ausmachen konnte, ob Stress additive genetische Varianz (V_A), Heritabilität (h^2), und das evolutionäre Potenzial *sensu* Houle (I_A) erhöht oder verringert, und ob der beobachtete Effekt auf die Neuheit oder Vertrautheit des jeweiligen Stressors zurückzuführen ist. Um diese Frage zu beantworten, zog ich Grasfroschkaulquappen mit unterschiedlichem genetischen Hintergrund in einem Mesokosmosexperiment auf, in dem ich vier verschiedene Stressoren manipulierte: erhöhter Salzgehalt und das Vorhandensein eines landwirtschaftlichen Pestizids (neuartige Stressoren), sowie Prädationsrisiko und hohe intraspezifische Konkurrenz (vertraute Stressoren). Mithilfe quantitativer genetischer Methoden konnte ich zeigen, dass neuartige stressige Bedingungen die additive genetische Varianz, die Heritabilität und das evolutionäre Potenzial *sensu* Houle verschiedener Merkmale erhöhen. Die Ergebnisse dieses Experiments legen nahe, dass die Merkmale, bei denen eine Erhöhung des evolutionären Potenzials unter Stress festgestellt werden konnte, relativ schnell mit einer adaptiven Antwort auf neuartige Stressoren reagieren können. Stressige Bedingungen, die nicht neuartig sind, sondern üblicherweise im natürlichen Lebensraum von *R. temporaria* vorkommen, führten zu keiner einheitlichen Veränderung von V_A, h^2 oder I_A. Das Ergebnis dieses Experiments legt nahe, dass mehrere Mechanismen erbliche Variation beeinflussen, und dass die Auslegung der Auswirkungen von Stress auf das evolutionäre Potential einer Population das Verständnis der Ökologie des jeweiligen Studienobjektes verlangt.

Kapitel 4

Fitness-trade-offs sind ein zentrales Konzept innerhalb der meisten Theorien für die Evolution ökologischer Spezialisierung. Trade-offs werden häufig zur Erklärung von Prozessen hinzugezogen, die ökologische Lebensgemeinschaften, die Verbreitung von Arten und die Spezialisierung auf bestimmte Lebensräume beeinflussen. Trade-offs können entstehen, wenn eine Art Merkmale ausbildet, die in einer Umgebungsbedingung relativ hohe Fitness gewährleistet, in einer anderen Umgebung allerdings zu relativ niedriger Fitness führt.

Es wird häufig angenommen, dass sich die Struktur ökologischer Lebensgemeinschaften, wie sie entlang eines Gradienten mit unterschiedlichen Dichten von Nahrungskonkurrenten und Fressfeinden vorkommt, durch einen trade-off zwischen der Konkurrenzfähigkeit und der Fähigkeit Fressfeinde zu vermeiden erklären lässt. Die Verbreitung einzelner Arten wird dann durch ihre relative Konkurrenzfähigkeit, oder ihre Fähigkeit, sich vor Fressfeinden zu verstecken, bestimmt.

In meinem vierten Kapitel habe ich untersucht, ob der durch Fressfeinde und Wettbewerb bestimmte trade-off auch innerhalb einer Art vorkommt. Ein trade-off würde sich dann zwischen Genotypen innerhalb derselben Population zeigen, die sich in ihrer relativen Konkurrenzfähigkeit, und ihrer Fähigkeit, Fressfeinde zu vermeiden, unterscheiden würden. Ausgehend von der Präsenz eines solchen trade-offs würde man davon ausgehen, dass die verschiedenen Genotypen innerhalb einer Population auf eine der beiden Fähigkeiten spezialisiert sind. Das würde sich wiederum als eine genetische Korrelation der mit diesen Fähigkeiten assoziierten Merkmale zwischen den verschiedenen Umweltbedingungen äussern. Relative Fitness und Aktivität sind diejenigen Merkmale, die beim Grasfrosch einen trade-off zwischen den zwei genannten Umweltbedingungen ausbilden können.

Um die Existenz eines trade-offs zu ermitteln, habe ich ein Mesokosmosexperiment durchgeführt. Dazu kreuzte ich erwachsene Grasfrösche und zog die Nachkommen jeder Familie unter verschiedenen Bedingungen auf, bei der das Umfeld jeder Familie bezüglich Prädationsrisiko und Dichte der Nahrungskonkurrenten manipuliert wurde. Die Ausprägung von Fitness, Aktivität und Merkmalen, die direkt mit Fitness assoziiert sind, wurden für jede Familie festgehalten und mit Hilfe quantitativ genetischer Methoden analysiert.

Ich konnte feststellen, dass drei der vier gemessenen Merkmale genetisch nicht korreliert sind, obwohl ich ein erhebliches Maß an additiver genetischer Varianz für die Mehrheit der Merkmale feststellen konnte. Körpergewicht war allerdings zwischen den zwei Umweltbedingungen positiv korreliert. Die Ergebnisse meiner Studie zeigen, dass Genotypen des Grasfrosches nicht darauf spezialisiert sind, entweder gute Konkurrenten oder gut bei der Vermeidung von Fressfeinden zu sein. Allerdings können die Ergebnisse meiner Studie die Möglichkeit nicht ausschließen, dass ein trade-off beim Grasfrosch existiert.

ACKNOWLEDGEMENTS

Acknowledgements

My first thanks go to Josh Van Buskirk, who supported me during this PhD project on so many levels: planning and realizing this project, helping me to analyze my data, and to write it all up to a comprehensive thesis. Thank you for giving up a field assistant back in 2009, and investing this money in the thesis in your hands. Thank you for your open doors, your discussions and insights, and for the freedom you gave me to develop and finish this project. Also, I would like to cordially thank you for introducing me to the world of polliwogs, which became truly dear to my heart.

I would like to thank Uli Reyer for accepting me as a PhD student, for his financial help, for providing the infrastructure and guiding the Ecology group through turbulent times. I would also like to thank the other members of my PhD committee, Wolf Blanckenorn and Katja Räsänen, for their support and valuable input for my project. If Katja would not have shared her knowledge with me, I would probably still be waiting for my experimental tadpole generation.

This PhD project would not have been possible without the financial support of the following institutions and foundations: Australian Research Council, Promotorstiftung, and Schweizerischer Nationalfonds.

I thank the Ecology group for welcoming me so warmly back in 2009, and for their steady support ever since. I always appreciated the friendly and fun atmosphere that could also be gravely scientific, whenever needed. My thanks go to all current and past members of the Ecology group for the wonderful time during the last years.

Many thanks go to Anni Mäder, Susanne Bischof and Isabel Schöchli who kept and are keeping everything running behind the scenes. I also thank Michel Nakano und Tina Siegenthaler for dealing with my recurrent computer problems.

A lot of people helped me in the field, and I would like to thank Corina Geiger, Ralf Jochmann, Morana Mihaljević, Oscar Ramos, Lorenzo Tanadini, Ursina Tobler, Josh Van Buskirk, Lukas Winkler, and Iris Wurmitzer for their commitment. Without you, my large-scale experiment would have become a large-scale disaster.

A large number of people from the IEU and other institutions supported me in all conceivable ways, be it through discussions, help with my analyses, solving logistical problems, or other forms of support. I cordially thank Alice Balmer, Andrew Beckerman, Lisa Bitterlin, Glauco Camenisch, Leyla Davis, Corina Geiger, Marta Giordano, Ralf Hendrix, Alexandra Hoffmann, Martina Keller, Jenny Morger, Owen Petchey and the R lunch group, Gian-Marco Palamara, Marco Plebani, Erik Postma, Nicolas Pruvost, Oscar Ramos, Sylvia Rauch, Sandy Röthlisberger, Martin Schäfer, Benedikt Schmidt, Lorenzo Tanadini, Matteo Tanadini, Ursina Tobler, Barbara Tschirren, Alex Wegmann, and Camilla Whittington.

For their friendship, support and humor during those last years, I would like to thank Iris Eigenmann, Corina Geiger, Valeria Rispoli, Ursina Tobler and Alex Wegmann. Thank you for keeping up my spirit! Your aid was badly needed many times, and I could always count on you.

I cordially thank my family for their support, and for their understanding for my choice of work and life. I am grateful to have a family that always encouraged me to do what I wanted to do, and to go out and discover this world.

My special thanks go to Lorenzo, who supported me in truly all conceivable ways, and for his faith in me.

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